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FILE 'HOME' ENTERED AT 08:23:32 ON 09 JAN 2002
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e menozzi franco/au
E1      121   MENOZZI F D/AU
E2        1   MENOZZI FRANCE O/AU
E3        6 --> MENOZZI FRANCO/AU
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E5      178   MENOZZI G/AU
E6        1   MENOZZI GABRIELLA NEBBIA/AU
E7       65   MENOZZI GIULIA/AU
E8        3   MENOZZI GIUSEPPE/AU
E9       24   MENOZZI I/AU
E10       1   MENOZZI ITALO/AU
E11       1   MENOZZI JAY P/AU
E12      70   MENOZZI L/AU
=> s e1-e4 and mycobact?
L1       38 ("MENOZZI F D"/AU OR "MENOZZI FRANCE O"/AU OR "MENOZZI FRANCO"/A
           U OR "MENOZZI FRANCO D"/AU) AND MYCOBACT?
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2       15 DUP REM L1 (23 DUPLICATES REMOVED)
=> d bib ab 1-
L2  ANSWER 1 OF 15  SCISEARCH  COPYRIGHT 2002 ISI (R)
AN  2002:9648  SCISEARCH
GA  The Genuine Article (R) Number: 501HY
TI  An adhesin responsible for disemination of  ***Mycobacterium***
    tuberculosis
AU  Pethe K (Reprint);  ***Menozzi F D***  ; Locht C
CS  Inst Pasteur, Inserm U447, 1 Rue Prof Calmette, Lille, France (Reprint);
    Inst Pasteur, Inserm U447, Lille, France
CYA  France
SO  M S-MEDECINE SCIENCES, (NOV 2001) Vol. 17, No. 11, pp. 1220-1221.
    Publisher: MASSON EDITEUR, 120 BLVD SAINT-GERMAIN, 75280 PARIS 06, FRANCE.
    ISSN: 0767-0974.
DT  News Announcement; Journal
LA  French
REC  Reference Count: 11

L2  ANSWER 2 OF 15  BIOSIS  COPYRIGHT 2002 BIOSIS     DUPLICATE 1
AN  2001:528989  BIOSIS
DN  PREV200100528989
TI  The heparin-binding haemagglutinin of M. tuberculosis is required for
    extrapulmonary dissemination.
AU  Pethe, Kevin; Alonso, Sylvie; Biet, Franck; Delogu, Giovanni; Brennan,
    Michael J.; Locht, Camille (1);  ***Menozzi, Franco D.***
CS  (1) Unite INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette,
    F-59019, Lille Cedex: camille.locht@pasteur.lille.fr France
SO  Nature (London), (12 July, 2001) Vol. 412, No. 6843, pp. 190-194. print.
    ISSN: 0028-0836.
DT  Article
LA  English
SL  English
AB  Tuberculosis remains the world's leading cause of death due to a single
    infectious agent,  ***Mycobacterium***  tuberculosis, with 3 million
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deaths and 10 million new cases per year. The infection initiates in the lungs and can then spread rapidly to other tissues. The availability of the entire M. tuberculosis genome sequence and advances in gene disruption technologies have led to the identification of several ***mycobacterial*** determinants involved in virulence. However, no virulence factor specifically involved in the extrapulmonary dissemination of M. tuberculosis has been identified to date. Here we show that the disruption of the M. tuberculosis or ***Mycobacterium*** bovis Bacille Calmette-Guerin (BCG) hbhA gene encoding the heparin-binding haemagglutinin adhesin (HBHA) markedly affects ***mycobacterial*** interactions with epithelial cells, but not with macrophage-like cells. When nasally administered to mice, the mutant strains were severely impaired in spleen colonization, but not in lung colonization. Coating wild-type ***mycobacteria*** with anti-HBHA antibodies also impaired dissemination after intranasal infection. These results provide evidence that adhesins such as HBHA are required for extrapulmonary dissemination, and that interactions with non-phagocytic cells have an important role in the pathogenesis of tuberculosis. They also suggest that antibody responses to HBHA may add to immune protection against tuberculosis.

L2 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 2
AN 2001:71458 BIOSIS
DN PREV200100071458
TI ***Mycobacterium*** smegmatis laminin-binding glycoprotein shares epitopes with ***Mycobacterium*** tuberculosis heparin-binding haemagglutinin.
AU Pethe, Kevin; Puech, Virginie; Daffe, Mamadou; Josenhans, Christine; Drobecq, Herve; Locht, Camille; ***Menozzi, Franco D. (1)***
CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, 1 Rue A. Calmette, 59019, Lille Cedex: franco.menozzi@pasteur-lille.fr France
SO Molecular Microbiology, (January, 2001) Vol. 39, No. 1, pp. 89-99. print. ISSN: 0950-382X.
DT Article
LA English
SL English
AB ***Mycobacterium*** tuberculosis, the causative agent of tuberculosis, produces a heparin-binding haemagglutinin adhesin (HBHA), which is involved in its epithelial adherence. To ascertain whether HBHA is also present in fast-growing ***mycobacteria*** , ***Mycobacterium*** smegmatis was studied using anti-HBHA monoclonal antibodies (mAbs). A cross-reactive protein was detected by immunoblotting of M. smegmatis whole-cell lysates. However, the M. tuberculosis HBHA-encoding gene failed to hybridize with M. smegmatis chromosomal DNA in Southern blot analyses. The M. smegmatis protein recognized by the anti-HBHA mAbs was purified by heparin-Sepharose chromatography, and its amino-terminal sequence was found to be identical to that of the previously described histone-like protein, indicating that M. smegmatis does not produce HBHA. Biochemical analysis of the M. smegmatis histone-like protein shows that it is glycosylated like HBHA. Immunoelectron microscopy demonstrated that the M. smegmatis protein is present on the ***mycobacterial*** surface, a cellular localization inconsistent with a histone-like function, but compatible with an adhesin activity. In vitro protein interaction assays showed that this glycoprotein binds to laminin, a major component of basement membranes. Therefore, the protein was called M. smegmatis

laminin-binding protein (MS-LBP). MS-LBP does not appear to be involved in adherence in the absence of laminin but is responsible for the laminin-mediated ***mycobacterial*** adherence to human pneumocytes and macrophages. Homologous laminin-binding adhesins are also produced by virulent ***mycobacteria*** such as M. tuberculosis and ***Mycobacterium*** leprae, suggesting that this adherence mechanism may contribute to the pathogenesis of ***mycobacterial*** diseases.

L2 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 2000:814638 CAPLUS
DN 133:359789
TI Affinity purification of recombinant proteins
IN ***Menozzi, Franco*** ; Locht, Camille; Pethe, Kevin
PA Institut Pasteur de Lille, Fr.; Institut National de la Sante et de la Recherche Medicale
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000068398	A1	20001116	WO 2000-FR1282	20000511
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
FR 2793492	A1	20001117	FR 1999-6031	19990511
PRAI FR 1999-6031	A	19990511		

OS MARPAT 133:359789
AB The invention concerns a novel method for producing and purifying a protein of interest by affinity chromatog. on a solid support bearing sulfated polysaccharides. Thus, the protein is fused at the N- or C-terminus with a label consisting of .gtoreq.1 R1 motifs of formula X1X2Y1Y2PY3 and 0-5 R2 motifs of formula X1X2Y1Y2Y3X3X4 (X1-X4 = Lys or Arg; Y1-Y3 = any amino acid, but preferably Ala, Leu, Ileu, or Val, and P = proline; the no. of R1 and R2 motifs and their combination being selected on the basis of the desired affinity between the polypeptide and the solid support bearing sulfated polysaccharides, provided that when R2 = 0, R1 .gtoreq. 2). Thus, ***Mycobacterium*** tuberculosis heparin-binding hemagglutinin was prepd. with recombinant, protease-deficient E. coli. The hemagglutinin could be affinity purified using heparin-Sepharose. The affinity of the hemagglutinin for the affinity matrix was dependent on the presence of lysine-rich repeat motifs in the C-terminus of the hemagglutinin. Modifying the no. of these repeat units modified the affinity of the hemagglutinin for the heparin-Sepharose.
RE.CNT 6
RE
(1) Cardin, A; ARTERIOSCLEROSIS V9(1), P21 CAPLUS

(2) Kagaku Oyobi Kessei Ryoho; JP 61054451 A 1986 CAPLUS
(3) Menozzi, F; WO 9744463 A 1997 CAPLUS
(4) Menozzi, F; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1998, V95(21), P12625 CAPLUS
(5) Pasteur Institut; WO 9528486 A 1995 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 3
AN 2000:345156 BIOSIS
DN PREV200000345156
TI Characterization of the heparin-binding site of the ***mycobacterial*** heparin-binding hemagglutinin adhesin.
AU Pethe, Kevin; Aumercier, Marc; Fort, Emmanuelle; Gatot, Christophe; Locht, Camille (1); ***Menozzi, Franco D.***
CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, Institut de Biologie de Lille, 1 Rue A. Calmette, 59019, Lille Cedex France
SO Journal of Biological Chemistry, (May 12, 2000) Vol. 275, No. 19, pp. 14273-14280. print.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB The ***mycobacterial*** adhesin heparin-binding hemagglutinin (HBHA) contains several lysine-rich repeats at its carboxyl-terminal end. Using truncated recombinant HBHA forms and hybrid proteins containing HBHA repeats grafted onto the Escherichia coli maltose-binding protein (MBP), we found that these repeats are responsible for heparin binding. Immunofluorescence microscopy studies revealed that their deletion abrogates binding of HBHA to human pneumocytes. Conversely, when fused to MBP, the HBHA repeats confer pneumocyte adherence properties to the hybrid protein. Treatment of pneumocytes with glycosaminoglycan-degrading enzymes showed that HBHA binding depends on the presence of heparan sulfate chains on the cell surface. The epitope of a monoclonal antibody that inhibits ***mycobacterial*** adherence to epithelial cells was mapped within the lysine-rich repeats, confirming their involvement in ***mycobacterial*** adherence to epithelial cells. Surface plasmon resonance analyses showed that recombinant HBHA binds to immobilized heparin with fast association kinetics (Ka = 5.62 (+- 0.10) X 105 M-1 s-1), whereas the dissociation kinetics were slower (kd = 0.015 (+- 0.002) s-1), yielding a KD value of 26 nM. Similar analyses with grafted MBP indicated similar kinetic constants, indicating that the carboxyl-terminal repeats contain the entire heparin-binding site of HBHA. The molecular characterization of the interactions of HBHA with epithelial glycosaminoglycans should help to better understand ***mycobacterial*** adherence within the lungs and may ultimately lead to new approaches for therapy or immunoprophylaxis.

L2 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1999:342091 BIOSIS
DN PREV199900342091
TI Functional and immunological characterizations of the lysine-rich repeats in the C-terminal domain of the ***mycobacterial*** heparin-binding hemagglutinin.
AU Pethe, K. (1); Locht, C. (1); ***Menozzi, F. D. (1)***
CS (1) INSERM U447 Institut Pasteur, Lille France

SO Abstracts of the General Meeting of the American Society for Microbiology, (1999) Vol. 99, pp. 654.
Meeting Info.: 99th General Meeting of the American Society for Microbiology Chicago, Illinois, USA May 30-June 3, 1999 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English

L2 ANSWER 7 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 4
AN 1998:497675 BIOSIS
DN PREV199800497675
TI Molecular characterization of the ***mycobacterial*** heparin-binding hemagglutinin, a ***mycobacterial*** adhesin.
AU ***Menozzi, Franco D.*** ; Bischoff, Rainer; Fort, Emmanuelle; Brennan, Michael J.; Locht, Camile (1)
CS (1) Lab. Microbiol., Genet. Mol., Inst. Natl. Sante Recherche Med. U447, Inst. Pasteur Lille, 1 rue Calmette, F-59019 Lille Cedex France
SO Proceedings of the National Academy of Sciences of the United States of America, (Oct. 13, 1998) Vol. 95, No. 21, pp. 12625-12630.
ISSN: 0027-8424.
DT Article
LA English
AB Although it generally is accepted that the interaction of ***Mycobacterium*** tuberculosis with alveolar macrophages is a key step in the pathogenesis of tuberculosis, interactions with other cell types, especially epithelia(cells, also may be important. In this study we describe the molecular characterization of a ***mycobacterial*** heparin-binding hemagglutinin (HBHA), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from M. tuberculosis and bacillus Calmette-Guerin, and the sequence was found to be identical between the two species. The calculated M, was smaller than the observed Mr when analyzed by SDS/PAGE. This difference can be attributed to the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of HBHA appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of HBHA to sulfated carbohydrates. Evidence suggests that glycosylation is also important for HBHA-mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of HBHA raises the possibility that this protein is not secreted via the general secretion pathway.

L2 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1997:776266 CAPLUS
DN 128:58314
TI Cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection
IN ***Menozzi, Franco*** ; Locht, Camille
PA Institut Pasteur De Lille, Fr.; Institut National De La Sante Et De La Recherche Medicale; Menozzi, Franco; Locht, Camille
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent

LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9744463	A2	19971127	WO 1997-FR886	19970520
WO 9744463	A3	19980129		
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2748748	A1	19971121	FR 1996-6168	19960517
FR 2748748	B1	19981106		
AU 9730362	A1	19971209	AU 1997-30362	19970520
EP 914437	A2	19990512	EP 1997-925109	19970520
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI FR 1996-6168 19960517
WO 1997-FR886 19970520

AB The invention features proteins enabling ***mycobacteria*** adhesion to host cells, in particular to epithelial cells. More particularly, the invention features a ***mycobacterial*** antigen of the heparin-binding hemagglutinin type (HBHA) obtained from ***Mycobacterium*** bovis BCG or M. tuberculosis. The invention particularly features the expression product of an Escherichia coli strain transformed with an HBHA gene. These protein sequences can be used in immunogenic compns., for prepg. vaccines against ***mycobacterial*** infections, and for diagnosis of ***mycobacterial*** infection. Addnl., use of sulfated carbohydrates (such as heparin, chondroitin sulfate, dextran sulfate) to prevent adhesion of ***mycobacteria*** to epithelial cells is claimed. Sera of tuberculosis patients contain antibodies to HBHA while sera of healthy people do not. The post-translational modification of native HBHA (presumably glycosylation) was shown to provide a major antigenic determinant of HBHA.

L2 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1998:88157 CAPLUS
DN 128:99777
TI A heparin-binding hemagglutinin of ***Mycobacterium*** for use as an antigen and the gene encoding it
IN ***Menozzi, Franco*** ; Locht, Camille
PA Institut Pasteur De Lille, Fr.; Institut National de la Sante et de la Recherche Medicale (INSERM)
SO Fr. Demande, 50 pp.
CODEN: FRXXBL
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI FR 2748749	A1	19971121	FR 1996-6169	19960517

AB An adhesion protein of ***Mycobacterium*** responsible for binding to epithelial cells of a host is identified and the gene encoding it is cloned. The protein may be of use as an antigen, or as a target for adhesion-inhibiting drugs. Binding of ***Mycobacterium*** to epithelial cells was inhibited by sulfated polysaccharides such as heparin and chondroitin sulfate, but not by sugars such as galactose. A 28 kDa heparin-binding protein was purified from ***Mycobacterium*** bovis

BCG by affinity chromatog. against heparin-Sepharose. The gene was cloned by PCR.

L2 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1997:284755 BIOSIS
DN PREV199799583958
TI A new type of repetitive element in ***mycobacterial*** two-component system operons.
AU Supply, P.; Magdalena, J.; Himpens, S.; ***Menozzi, F. D.*** ; Locht, C.
CS INSERM U447, Inst. Pasteur de Lille, Lille France
SO Abstracts of the General Meeting of the American Society for Microbiology, (1997) Vol. 97, No. 0, pp. 551.
Meeting Info.: 97th General Meeting of the American Society for Microbiology Miami Beach, Florida, USA May 4-8, 1997
ISSN: 1060-2011.
DT Conference; Abstract; Conference
LA English

L2 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1998:112510 BIOSIS
DN PREV199800112510
TI Isolation, gene cloning and partial characterization of a novel heparin-binding protein produced by ***mycobacteria*** .
AU ***Menozzi, F. D. (1)*** ; Locht, C.
CS (1) Inst. Pasteur de Lille, 1 Rue Calmette, Unite INSERM U447, Lille 59021 France
SO Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (1997) Vol. 37, pp. 32.
Meeting Info.: 37th Interscience Conference on Antimicrobial Agents and Chemotherapy Toronto, Ontario, Canada September 28-October 1, 1997 ICAAC
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DT Conference
LA English

L2 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 5
AN 1996:510320 BIOSIS
DN PREV199699232676
TI Identification of a heparin-binding hemagglutinin present in ***mycobacteria*** .
AU ***Menozzi, Franco D.*** ; Rouse, Julie H.; Alavi, Mohammad; Laude-Sharp, Marilyn; Muller, Jacqueline; Bischoff, Rainer; Brennan, Michael J. (1); Locht, Camille
CS (1) CBER/FDA, 1401 Rockville Pike (HFM-431), Rockville, MD 20852-1448 USA
SO Journal of Experimental Medicine, (1996) Vol. 184, No. 3, pp. 993-1001.
ISSN: 0022-1007.
DT Article
LA English
AB Adherence to mammalian host tissues is an important virulence trait in microbial pathogenesis, yet little is known about the adherence mechanisms of ***mycobacteria*** . Here, we show that binding of ***mycobacteria*** to epithelial cells but not to macrophages can be specifically inhibited by sulfated carbohydrates. Using heparin-Sepharose chromatography, a 28-kD heparin-binding protein was purified from culture supernatants and cell extracts of ***Mycobacterium*** bovis and

Mycobacterium tuberculosis. This protein, designated heparin-binding hemagglutinin (HBHA), promotes the agglutination of rabbit erythrocytes, which is specifically inhibited by sulfated carbohydrates. HBHA also induces ***mycobacterial*** aggregation, suggesting that it can mediate bacteria-bacteria interactions as well. Hemagglutination, ***mycobacterial*** aggregation, as well as attachment to epithelial cells are specifically inhibited in the presence of anti-HBHA antibodies. Immunoelectron microscopy using anti-HBHA monoclonal antibodies revealed that the protein is surface exposed, consistent with a role in adherence. Immunoblot analyses using antigen-specific antibodies indicated that HBHA is different from the fibronectin-binding proteins of the antigen 85 complex and p55, and comparison of the NH-2-terminal amino acid sequence of purified HBHA with the protein sequence data bases did not reveal any significant similarity with other known proteins. Sera from tuberculosis patients but not from healthy individuals were found to recognize HBHA, indicating its immunogenicity in humans during ***mycobacterial*** infections. Identification of putative ***mycobacterial*** adhesins, such as the one described in this report, may provide the basis for the development of new therapeutic and prophylactic strategies against ***mycobacterial*** diseases.

L2 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1996:259145 BIOSIS
DN PREV199698815274
TI Molecular characterization of a two-component system of ***Mycobacterium*** bovis BCG and ***Mycobacterium*** tuberculosis.
AU Supply, P.; Himpens, S.; ***Menozzi, F. D.*** ; Locht, C.
CS Inst. Pasteur de Lille France
SO Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 131.
Meeting Info.: 96th General Meeting of the American Society for Microbiology New Orleans, Louisiana, USA May 19-23, 1996
ISSN: 1060-2011.
DT Conference
LA English

L2 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1995:290427 BIOSIS
DN PREV199598304727
TI A heparin-binding hemagglutinin in ***mycobacteria*** .
AU ***Menozzi, F. D. (1)*** ; Rouse, J. H.; Laud-Sharp, M.; Brennan, M. J.; Locht, C. (1)
CS (1) Inst. Pasteur, Lille France
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 193.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference
LA English

L2 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1995:147915 BIOSIS
DN PREV199598162215
TI Identification of a heparin-binding ***mycobacterial*** hemagglutinin.

AU Brennan, Michael J. (1); Laude-Sharp, Maryline (1); Hannah, Julie H. (1); ***Menozzi, Franco*** ; Locht, Camille
CS (1) DBP, CBER, FDA, Bethesda, MD 20852 USA
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 66.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis Tamarron, Colorado, USA February 19-25, 1995
ISSN: 0733-1959.
DT Conference
LA English

=> e locht camille/au
E1 1 LOCHT CAMILE/AU
E2 1 LOCHT CAMILL/AU
E3 167 --> LOCHT CAMILLE/AU
E4 1 LOCHT E/AU
E5 1 LOCHT GUILLERMO/AU
E6 1 LOCHT GUILLERMO A/AU
E7 36 LOCHT H/AU
E8 2 LOCHT H J VAN DER/AU
E9 8 LOCHT HENNING/AU
E10 1 LOCHT J L/AU
E11 1 LOCHT JEAN LUC/AU
E12 4 LOCHT L J/AU

=> s e1-e3 and mycobact?
L3 58 ("LOCHT CAMILE"/AU OR "LOCHT CAMILL"/AU OR "LOCHT CAMILLE"/AU) AND MYCOBACT?

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 34 DUP REM L3 (24 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 34 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 1
AN 2001:420421 BIOSIS
DN PREV200100420421
TI Biochemical characterization of acyl carrier protein (AcpM) and malonyl-CoA:AcpM transacylase (mtFabD), two major components of ***Mycobacterium*** tuberculosis fatty acid synthase II.
AU Kremer, Laurent; Nampoothiri, K. Madhavan; Lesjean, Sarah; Dover, Lynn G.; Graham, Steven; Betts, Joanna; Brennan, Patrick J.; Minnikin, David E.; ***Locht, Camille*** ; Besra, Gurdyal S. (1)
CS (1) Department of Microbiology and Immunology, University of Newcastle, Newcastle upon Tyne, NE2 4HH: g.s.besra@newcastle.ac.uk UK
SO Journal of Biological Chemistry, (July 27, 2001) Vol. 276, No. 30, pp. 27967-27974. print.
ISSN: 0021-9258.
DT Article
LA English
SL English

AB Malonyl coenzyme A (CoA)-acyl carrier protein (ACP) transacylase (MCAT) is an essential enzyme in the biosynthesis of fatty acids in all bacteria, including ***Mycobacterium*** tuberculosis. MCAT catalyzes the transacylation of malonate from malonyl-CoA to activated holo-ACP, to generate malonyl-ACP, which is an elongation substrate in fatty acid biosynthesis. To clarify the roles of the ***mycobacterial*** acyl carrier protein (AcpM) and MCAT in fatty acid and mycolic acid biosynthesis, we have cloned, expressed, and purified acpM and mtfabD (malonyl-CoA:AcpM transacylase) from M. tuberculosis. According to the culture conditions used, AcpM was produced in Escherichia coli in two or three different forms: apo-AcpM, holo-AcpM, and palmitoylated-AcpM, as revealed by electrospray mass spectrometry. The mtfabD gene encoding a putative MCAT was used to complement a thermosensitive E. coli fabD mutant. Expression and purification of mtFabD resulted in an active enzyme displaying strong MCAT activity in vitro. Enzymatic studies using different ACP substrates established that holo-AcpM constitutes the preferred substrate for mtFabD. In order to provide further insight into the structure-function relationship of mtFabD, different mutant proteins were generated. All mutations (Q9A, R116A, H194A, Q243A, S91T, and S91A) completely abrogated MCAT activity in vitro, thus underlining the importance of these residues in transacylation. The generation and characterization of the AcpM forms and mtFabD opens the way for further studies relating to fatty acid and mycolic acid biosynthesis to be explored in M. tuberculosis. Since a specific type of FabD is found in ***mycobacterial*** species, it represents an attractive new drug target waiting to be exploited.

L4 ANSWER 2 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 2
AN 2001:543083 BIOSIS
DN PREV200100543083
TI Automated high-throughput genotyping for study of global epidemiology of ***Mycobacterium*** tuberculosis based on ***mycobacterial*** interspersed repetitive units.
AU Supply, Philip (1); Lesjean, Sarah; Savine, Evgueni; Kremer, Kristin; van Soolingen, Dick; ***Locht, Camille***
CS (1) Laboratoire des Mecanismes Moleculaires de la Pathogenese Bacterienne, INSERM U447, Institut Pasteur de Lille, 1, Rue du Prof. Calmette, F-59019, Lille Cedex: Philip.Supply@pasteur-lille.fr France
SO Journal of Clinical Microbiology, (October, 2001) Vol. 39, No. 10, pp. 3563-3571. print.
ISSN: 0095-1137.
DT Article
LA English
SL English
AB Large-scale genotyping of ***Mycobacterium*** tuberculosis is especially challenging, as the current typing methods are labor-intensive and the results are difficult to compare among laboratories. Here, automated typing based on variable-number tandem repeats (VNTRs) of genetic elements named ***mycobacterial*** interspersed repetitive units (MIRUs) in 12 mammalian minisatellite-like loci of M. tuberculosis is presented. This system combines analysis of multiplex PCRs on a fluorescence-based DNA analyzer with computerized automation of the genotyping. Analysis of a blinded reference set of 90 strains from 38 countries (K. Kremer et al., J. Clin. Microbiol. 37:2607-2618, 1999) demonstrated that it is 100% reproducible, sensitive, and specific for M.

tuberculosis complex isolates, a performance that has not been achieved by any other typing method tested in the same conditions. MIRU-VNTRs can be used for analysis of the global genetic diversity of M. tuberculosis complex strains at different levels of evolutionary divergence. To fully exploit the portability of this typing system, a website was set up for the analysis of M. tuberculosis MIRU-VNTR genotypes via the Internet. This opens the way for global epidemiological surveillance of tuberculosis and should lead to novel insights into the evolutionary and population genetics of this major pathogen.

L4 ANSWER 3 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 3
AN 2001:176780 BIOSIS
DN PREV200100176780
TI High-resolution minisatellite-based typing as a portable approach to global analysis of ***Mycobacterium*** tuberculosis molecular epidemiology.
AU Mazars, Edith; Lesjean, Sarah; Banuls, Anne-Laure; Gilbert, Michele; Vincent, Veronique; Gicquel, Brigitte; Tibayrenc, Michel; ***Locht,*** *** Camille*** ; Supply, Philip (1)
CS (1) Laboratoire des Mecanismes Moleculaires de la Pathogenese Bacterienne, Institut National de la Sante et de la Recherche Medicale, U447, Institut Pasteur de Lille, 1, Rue du Prof. Calmette, F-59019, Lille Cedex: philip.supply@pasteur-lille.fr France
SO Proceedings of the National Academy of Sciences of the United States of America, (February 13, 2001) Vol. 98, No. 4, pp. 1901-1906. print. ISSN: 0027-8424.
DT Article
LA English
SL English
AB The worldwide threat of tuberculosis to human health emphasizes the need to develop novel approaches to a global epidemiological surveillance. The current standard for ***Mycobacterium*** tuberculosis typing based on IS6110 restriction fragment length polymorphism (RFLP) suffers from the difficulty of comparing data between independent laboratories. Here, we propose a high-resolution typing method based on variable number tandem repeats (VNTRs) of genetic elements named ***mycobacterial*** interspersed repetitive units (MIRUs) in 12 human minisatellite-like regions of the M. tuberculosis genome. MIRU-VNTR profiles of 72 different M. tuberculosis isolates were established by PCR analysis of all 12 loci. From 2 to 8 MIRU-VNTR alleles were identified in the 12 regions in these strains, which corresponds to a potential of over 16 million different combinations, yielding a resolution power close to that of IS6110-RFLP. All epidemiologically related isolates tested were perfectly clustered by MIRU-VNTR typing, indicating that the stability of these MIRU-VNTRs is adequate to track outbreak episodes. The correlation between genetic relationships inferred from MIRU-VNTR and IS6110-RFLP typing was highly significant. Compared with IS6110-RFLP, high-resolution MIRU-VNTR typing has the considerable advantages of being fast, appropriate for all M. tuberculosis isolates, including strains that have a few IS6110 copies, and permitting easy and rapid comparison of results from independent laboratories. This typing method opens the way to the construction of digital global databases for molecular epidemiology studies of M. tuberculosis.

L4 ANSWER 4 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 4

AN 2001:528989 BIOSIS
DN PREV200100528989
TI The heparin-binding haemagglutinin of M. tuberculosis is required for extrapulmonary dissemination.
AU Pethe, Kevin; Alonso, Sylvie; Biet, Franck; Delogu, Giovanni; Brennan, Michael J.; ***Locht, Camille (1)*** ; Menozzi, Franco D.
CS (1) Unite INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette, F-59019, Lille Cedex: camille.locht@pasteur.lille.fr France
SO Nature (London), (12 July, 2001) Vol. 412, No. 6843, pp. 190-194. print. ISSN: 0028-0836.
DT Article
LA English
SL English
AB Tuberculosis remains the world's leading cause of death due to a single infectious agent, ***Mycobacterium*** tuberculosis, with 3 million deaths and 10 million new cases per year. The infection initiates in the lungs and can then spread rapidly to other tissues. The availability of the entire M. tuberculosis genome sequence and advances in gene disruption technologies have led to the identification of several ***mycobacterial*** determinants involved in virulence. However, no virulence factor specifically involved in the extrapulmonary dissemination of M. tuberculosis has been identified to date. Here we show that the disruption of the M. tuberculosis or ***Mycobacterium*** bovis Bacille Calmette-Guerin (BCG) hbhA gene encoding the heparin-binding haemagglutinin adhesin (HBHA) markedly affects ***mycobacterial*** interactions with epithelial cells, but not with macrophage-like cells. When nasally administered to mice, the mutant strains were severely impaired in spleen colonization, but not in lung colonization. Coating wild-type ***mycobacteria*** with anti-HBHA antibodies also impaired dissemination after intranasal infection. These results provide evidence that adhesins such as HBHA are required for extrapulmonary dissemination, and that interactions with non-phagocytic cells have an important role in the pathogenesis of tuberculosis. They also suggest that antibody responses to HBHA may add to immune protection against tuberculosis.

L4 ANSWER 5 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 5
AN 2001:71458 BIOSIS
DN PREV200100071458
TI ***Mycobacterium*** smegmatis laminin-binding glycoprotein shares epitopes with ***Mycobacterium*** tuberculosis heparin-binding haemagglutinin.
AU Pethe, Kevin; Puech, Virginie; Daffe, Mamadou; Josenhans, Christine; Drobecq, Herve; ***Locht, Camille*** ; Menozzi, Franco D. (1)
CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, 1 Rue A. Calmette, 59019, Lille Cedex: franco.menozzi@pasteur-lille.fr France
SO Molecular Microbiology, (January, 2001) Vol. 39, No. 1, pp. 89-99. print. ISSN: 0950-382X.
DT Article
LA English
SL English
AB ***Mycobacterium*** tuberculosis, the causative agent of tuberculosis, produces a heparin-binding haemagglutinin adhesin (HBHA), which is involved in its epithelial adherence. To ascertain whether HBHA is also present in fast-growing ***mycobacteria*** , ***Mycobacterium***

smegmatis was studied using anti-HBHA monoclonal antibodies (mAbs). A cross-reactive protein was detected by immunoblotting of M. smegmatis whole-cell lysates. However, the M. tuberculosis HBHA-encoding gene failed to hybridize with M. smegmatis chromosomal DNA in Southern blot analyses. The M. smegmatis protein recognized by the anti-HBHA mAbs was purified by heparin-Sepharose chromatography, and its amino-terminal sequence was found to be identical to that of the previously described histone-like protein, indicating that M. smegmatis does not produce HBHA. Biochemical analysis of the M. smegmatis histone-like protein shows that it is glycosylated like HBHA. Immunoelectron microscopy demonstrated that the M. smegmatis protein is present on the ***mycobacterial*** surface, a cellular localization inconsistent with a histone-like function, but compatible with an adhesin activity. In vitro protein interaction assays showed that this glycoprotein binds to laminin, a major component of basement membranes. Therefore, the protein was called M. smegmatis laminin-binding protein (MS-LBP). MS-LBP does not appear to be involved in adherence in the absence of laminin but is responsible for the laminin-mediated ***mycobacterial*** adherence to human pneumocytes and macrophages. Homologous laminin-binding adhesins are also produced by virulent ***mycobacteria*** such as M. tuberculosis and ***Mycobacterium*** leprae, suggesting that this adherence mechanism may contribute to the pathogenesis of ***mycobacterial*** diseases.

L4 ANSWER 6 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 6
AN 2001:81590 BIOSIS
DN PREV200100081590
TI Shuttle vectors for the introduction of DNA into ***mycobacteria*** and utilization of such bacteria as vaccines.
AU Escuyer, Vincent (1); Baulard, Alain; Berche, Patrick; ***Locht,*** *** Camille*** ; Haddad, Nadia
CS (1) Massy France
ASSIGNEE: Institute National de la Sante et de la Recherche Medical (Inserm), Paris Cedex, France; Institut Pasteur de Lille, Lille Cedex, France
PI US 6074866 June 13, 2000
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 13, 2000) Vol. 1235, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133.
DT Patent
LA English
AB Shuttle vectors for inserting DNA in ***mycobacteria*** comprising at least one origin of functional replication in said ***mycobacteria*** , another origin of functional replication in other bacteria, an enzyme cutting site allowing the insertion of DNA coding for a protein capable of being expressed in the ***mycobacteria*** , characterized in that they also carry a gene providing on said ***mycobacteria*** resistance to a compound containing a heavy metal.

L4 ANSWER 7 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2000:814638 CAPLUS
DN 133:359789
TI Affinity purification of recombinant proteins
IN Menozzi, Franco; ***Locht, Camille*** ; Pethe, Kevin
PA Institut Pasteur de Lille, Fr.; Institut National de la Sante et de la Recherche Medicale

SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000068398	A1	20001116	WO 2000-FR1282	20000511
FR 2793492	A1	20001117	FR 1999-6031	19990511

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI FR 1999-6031 A 19990511
OS MARPAT 133:359789
AB The invention concerns a novel method for producing and purifying a protein of interest by affinity chromatog. on a solid support bearing sulfated polysaccharides. Thus, the protein is fused at the N- or C-terminus with a label consisting of .gtoreq.1 R1 motifs of formula X1X2Y1Y2PY3 and 0-5 R2 motifs of formula X1X2Y1Y2Y3X3X4 (X1-X4 = Lys or Arg; Y1-Y3 = any amino acid, but preferably Ala, Leu, Ileu, or Val, and P = proline; the no. of R1 and R2 motifs and their combination being selected on the basis of the desired affinity between the polypeptide and the solid support bearing sulfated polysaccharides, provided that when R2 = 0, R1 .gtoreq. 2). Thus, ***Mycobacterium*** tuberculosis heparin-binding hemagglutinin was prepd. with recombinant, protease-deficient E. coli. The hemagglutinin could be affinity purified using heparin-Sepharose. The affinity of the hemagglutinin for the affinity matrix was dependent on the presence of lysine-rich repeat motifs in the C-terminus of the hemagglutinin. Modifying the no. of these repeat units modified the affinity of the hemagglutinin for the heparin-Sepharose.
RE.CNT 6
RE
(1) Cardin, A; ARTERIOSCLEROSIS V9(1), P21 CAPLUS
(2) Kagaku Oyobi Kessei Ryoho; JP 61054451 A 1986 CAPLUS
(3) Menozzi, F; WO 9744463 A 1997 CAPLUS
(4) Menozzi, F; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1998, V95(21), P12625 CAPLUS
(5) Pasteur Institut; WO 9528486 A 1995 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2000:646770 CAPLUS
DN 133:319403
TI Activation of the pro-drug ethionamide is regulated in ***mycobacteria***
AU Baulard, Alain R.; Betts, Joanna C.; Engohang-Ndong, Jean; Quan, Selwyn; McAdam, Ruth A.; Brennan, Patrick J.; ***Locht, Camille*** ; Besra, Gurdyal S.

CS INSERM U447, Institut de Biologie de Lille, Institut Pasteur de Lille, Lille, 59019, Fr.
SO Journal of Biological Chemistry (2000), 275(36), 28326-28331
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB The anti-tuberculosis drug ethionamide (ETH), which is a structural analog of isoniazid (INH), is known to strongly inhibit mycolic acid synthesis in ***Mycobacterium*** tuberculosis. Although several targets have been identified for INH, only speculative information is available concerning ETH. Mutations within the promoter and the coding region of enoyl-acyl carrier protein reductase (InhA) were found to confer resistance to both drugs, thus leading to the impression that INH and ETH may share a common mode of action. However, a notable distinction between the two drugs lies in the lack of cross-resistance in clin. isolates. This may be attributed in part to the fact that the pro-drug INH must be activated via KatG, and no activation step for ETH has yet been described. Here, the authors report the identification of an activator for ETH. The ETH activator (Rv3854c), which was termed EthA, was found to be homologous to various monooxygenases, and it induced ETH sensitivity when overexpressed in ***mycobacteria*** . The neighboring open reading frame (Rv3855), which was homologous to transcriptional repressors of the tetR family, led to ETH resistance when overexpressed. In addn., chromosomal inactivation of this gene by transposition led to ETH hypersensitivity. These data strongly suggest that Rv3855, which was termed EthR, regulates the prodn. of EthA, which subsequently activates the pro-drug ETH. This study opens up new avenues of research relating to ETH activation in ***mycobacteria*** , possibly leading to an improved efficacy of ETH and to the generation of new anti- ***mycobacterial*** agents.
RE.CNT 38
RE
(1) Banerjee, A; Science 1994, V263, P227 CAPLUS
(2) Bardarov, S; Proc Natl Acad Sci U S A 1997, V94, P10961 CAPLUS
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(5) Beck, C; Microbiol Rev 1988, V52, P318 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 7
AN 2000:345156 BIOSIS
DN PREV200000345156
TI Characterization of the heparin-binding site of the ***mycobacterial*** heparin-binding hemagglutinin adhesin.
AU Pethe, Kevin; Aumercier, Marc; Fort, Emmanuelle; Gatot, Christophe; ***Locht, Camille (1)*** ; Menozzi, Franco D.
CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, Institut de Biologie de Lille, 1 Rue A. Calmette, 59019, Lille Cedex France
SO Journal of Biological Chemistry, (May 12, 2000) Vol. 275, No. 19, pp. 14273-14280. print.
ISSN: 0021-9258.
DT Article
LA English
SL English

AB The ***mycobacterial*** adhesin heparin-binding hemagglutinin (HBHA) contains several lysine-rich repeats at its carboxyl-terminal end. Using truncated recombinant HBHA forms and hybrid proteins containing HBHA repeats grafted onto the Escherichia coli maltose-binding protein (MBP), we found that these repeats are responsible for heparin binding. Immunofluorescence microscopy studies revealed that their deletion abrogates binding of HBHA to human pneumocytes. Conversely, when fused to MBP, the HBHA repeats confer pneumocyte adherence properties to the hybrid protein. Treatment of pneumocytes with glycosaminoglycan-degrading enzymes showed that HBHA binding depends on the presence of heparan sulfate chains on the cell surface. The epitope of a monoclonal antibody that inhibits ***mycobacterial*** adherence to epithelial cells was mapped within the lysine-rich repeats, confirming their involvement in ***mycobacterial*** adherence to epithelial cells. Surface plasmon resonance analyses showed that recombinant HBHA binds to immobilized heparin with fast association kinetics (Ka = 5.62 (+- 0.10) X 105 M-1 s-1), whereas the dissociation kinetics were slower (kd = 0.015 (+- 0.002) s-1), yielding a KD value of 26 nM. Similar analyses with grafted MBP indicated similar kinetic constants, indicating that the carboxyl-terminal repeats contain the entire heparin-binding site of HBHA. The molecular characterization of the interactions of HBHA with epithelial glycosaminoglycans should help to better understand ***mycobacterial*** adherence within the lungs and may ultimately lead to new approaches for therapy or immunoprophylaxis.

L4 ANSWER 10 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 8
AN 2000:349508 BIOSIS
DN PREV200000349508
TI Ineffective cellular immune response associated with T-cell apoptosis in susceptible ***Mycobacterium*** bovis BCG-infected mice.
AU Kremer, Laurent; Estaquier, Jerome (1); Wolowczuk, Isabelle; Biet, Franck; Ameisen, Jean-Claude; ***Locht, Camille***
CS (1) INSERM E9922, Groupe Hospitalier Bichat-Claude Bernard, 16 Rue Henri Huchard, 75018, Paris France
SO Infection and Immunity, (July, 2000) Vol. 68, No. 7, pp. 4264-4273. print. ISSN: 0019-9567.
DT Article
LA English
SL English
AB It has previously been reported that inhibition of delayed-type hypersensitivity-mediating functions of T cells during ***mycobacteria*** infection in mice is haplotype dependent. In the present study, we show that ***Mycobacterium*** bovis BCG infection induced, in susceptible C57BL/6 and BALB/c mice but not in resistant C3H/HeJ and DBA/2 mice, an important splenomegaly. An in vitro defect in T-cell proliferation in response to T-cell receptor (TCR) stimulation with mitogens or anti-CD3 antibodies was associated with enhanced levels of CD4+ and CD8+ T-cell apoptosis in susceptible but not in resistant mice 2 weeks after infection. Further investigations of C57BL/6 and C3H/HeJ mice revealed that in vivo splenomegaly was associated with destruction of the lymphoid tissue architecture, liver cellular infiltrates, and increased numbers of apoptotic cells in both spleen and liver tissue sections. Infection of C57BL/6 mice but not of C3H/HeJ mice induced massive production of tumor necrosis factor alpha (TNF-alpha) in serum, as well as an increase in Fas and Fas ligand (FasL) expression in T cells. In vitro addition of neutralizing anti-TNF-alpha antibodies led to a significant

reduction in CD3-induced T-cell apoptosis of both CD4+ and CD8+ T cells of C57BL/6 mice, while the blockade of Fas-FasL interactions reduced apoptosis only in CD4+ but not in CD8+ T cells. Together, these results suggest that TNF-alpha and Fas-FasL interactions play a role in the activation-induced cell death (AICD) process associated with a defect in T-cell proliferation of the susceptible C57BL/6 mice. T-cell death by apoptosis may represent one of the important components of the ineffective immune response against ***mycobacterium*** -induced immunopathology in susceptible hosts.

L4 ANSWER 11 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 9
AN 2001:49414 BIOSIS
DN PREV200100049414
TI Molecular characterization of the ***mycobacterial*** SenX3-RegX3 two-component system: Evidence for autoregulation.
AU Himpens, Sabine; ***Locht, Camille*** ; Supply, Philip (1)
CS (1) INSERM U447, Institut Pasteur de Lille/Institut de Biologie de Lille, 1 Rue du Professeur Calmette, F-59019, Lille Cedex: philip.supply@pasteur-lille.fr France
SO Microbiology (Reading), (December, 2000) Vol. 146, No. 12, pp. 3091-3098. print.
ISSN: 1350-0872.
DT Article
LA English
SL English
AB Environmental regulation of bacterial gene expression is often mediated by two-component signal transduction systems, which are themselves tightly regulated. The response regulator RegX3 and the cytoplasmic portion of the histidine kinase SenX3 from ***Mycobacterium*** bovis BCG were overproduced in Escherichia coli and purified as N-terminally (His)6-tagged proteins. Phosphorylation assays demonstrated autophosphorylation of the cytoplasmic portion of SenX3 and a phosphotransfer from SenX3 to RegX3, involving conserved histidine and aspartate residues, respectively. In addition, as shown by electrophoretic mobility shift assays, (His)6RegX3 was able to specifically bind to the promoter region of the senX3-regX3 operon. Furthermore, operon fusion analyses indicated that the overexpression of the senX3-regX3 operon increases the activity of the senX3 promoter in ***Mycobacterium*** smegmatis. Together, these results indicate that the ***mycobacterial*** SenX3-RegX3 two-component system is positively autoregulated.

L4 ANSWER 12 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 10
AN 2000:305446 BIOSIS
DN PREV200000305446
TI Variable human minisatellite-like regions in the ***Mycobacterium*** tuberculosis genome.
AU Supply, Philip (1); Mazars, Edith; Lesjean, Sarah; Vincent, Veronique; Gicquel, Brigitte; ***Locht, Camille***
CS (1) Laboratoire des Mecanismes Moleculaires de la Pathogenese Bacterienne, INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette, F-59019, Lille Cedex France
SO Molecular Microbiology, (May, 2000) Vol. 36, No. 3, pp. 762-771. print.
ISSN: 0950-382X.
DT Article
LA English

SL English
AB ***Mycobacterial*** interspersed repetitive units (MIRUs) are 40-100 bp DNA elements often found as tandem repeats and dispersed in intergenic regions of the ***Mycobacterium*** tuberculosis complex genomes. The M. tuberculosis H37Rv chromosome contains 41 MIRU loci. After polymerase chain reaction (PCR) and sequence analyses of these loci in 31 M. tuberculosis complex strains, 12 of them were found to display variations in tandem repeat copy numbers and, in most cases, sequence variations between repeat units as well. These features are reminiscent of those of certain human variable minisatellites. Of the 12 variable loci, only one was found to vary among genealogically distant BCG substrains, suggesting that these interspersed bacterial minisatellite-like structures evolve slowly in ***mycobacterial*** populations.

L4 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1999:589625 CAPLUS
DN 131:309666
TI In vivo immunomodulation following intradermal injection with DNA encoding IL-18
AU Kremer, Laurent; Dupre, Loic; Wolowczuk, Isabelle; ***Locht, Camille***
CS Moleculaire Institut National de la Sante et de la Recherche Medicale U447, Laboratoire de Microbiologie Genetique, Lille, Fr.
SO J. Immunol. (1999), 163(6), 3226-3231
CODEN: JOIMA3; ISSN: 0022-1767
PB American Association of Immunologists
DT Journal
LA English
AB IL-18, a recently identified cytokine synthesized by different cell types, including Kupffer cells, activated macrophages, and keratinocytes, induces IFN-.gamma. prodn. by T cells and NK cells. The cDNA encoding IL-18 with its natural signal peptide was cloned under control of the CMV promoter and injected into the skin of mice. A single intradermal injection of this construction led to efficient in vivo expression of IL-18 in cutaneous dermal cells and induced IFN-.gamma. mRNA prodn., indicating that it was produced in a biol. active form. In addn., a massive cellular infiltrate was obsd. in the skin 2 days after injection. When the mice were subsequently infected with ***Mycobacterium*** bovis bacillus Calmette-Guerin (BCG), they produced lower levels of anti-BCG Abs than control animals. However, in contrast to their lowered humoral immune response, the mice produced higher amts. of Ag-specific IFN-.gamma. after in vitro restimulation, as compared with the controls. Therefore, injection of DNA encoding IL-18 into the skin modulates both Ag-specific humoral and T cell responses upon ***mycobacterial*** infection. It increases the Th1 type response, which may be particularly useful for the development of new immunotherapeutic or immunoprotective approaches against infections by intracellular parasites, such as ***mycobacteria*** .
RE.CNT 37
RE
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(6) Cooper, A; J Exp Med 1997, V186, P39 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 14 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1999:150315 BIOSIS
DN PREV199900150315
TI Immunogenicity of recombinant BCG producing the GRA1 antigen from Toxoplasma gondii.
AU Supply, Philip; Sutton, Philip; Coughlan, Susie N.; Bilo, Katja; Saman, Eric; Trees, Alexander J.; Delauw, Marie-France Cesbron; ***Locht,*** *** Camille (1)***
CS (1) Lab. Microbiol. Genetique Mol., INSERM U447, Inst. Pasteur de Lille, 1 rue du Prof. Calmette, BP 245, 59019 Lille, Cedex France
SO Vaccine, (Feb., 1999) Vol. 17, No. 7-8, pp. 705-714.
ISSN: 0264-410X.
DT Article
LA English
AB Toxoplasmosis is a major parasitic disease, responsible for foetopathy in humans and domestic animals, especially sheep. Toxoplasma gondii infection generally protects immunocompetent hosts against subsequent reinfection, suggesting that efficacious vaccines can be developed against this disease. Excreted/secreted T. gondii antigens have previously been shown to provide immunoprotection in small rodents, and protective immunity is thought to be cell-mediated. ***Mycobacterium*** bovis BCG is known to be a good inducer of cellular immunity. In this study, we have developed a BCG strain which produces and secretes GRA1, one of the major excrete/disecreted T. gondii antigens. This strain does not carry antibiotic-resistance determinants and is therefore safe for the environment. The intraperitoneal immunization of OF1 outbred mice with this BCG strain failed to induce GRA1-specific humoral or cellular immune responses and only conferred a very limited degree of protection against challenge with virulent T. gondii. However, in sheep immunized subcutaneously and boosted intravenously, this recombinant BCG strain induced GRA1-specific cell-mediated responses, as evidenced by the proliferation of peripheral blood mononuclear cells and by the production of IFN-gamma, although it failed to elicit GRA1-specific antibody responses. Following oocyst challenge infection. sheep immunized with recombinant BCG exhibited an abbreviated temperature response compared with controls, suggesting partial protection.

L4 ANSWER 15 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1998:151234 CAPLUS
DN 128:201772
TI Sequences for identification of ***Mycobacterium*** tuberculosis complex members and their use in detection and differential diagnosis
IN Magdalena, Juana; Supply, Philip; ***Locht, Camille***
PA Institut Pasteur De Lille, Fr.; Institut National De La Sante Et De La Recherche Medicale (INSERM); Magdalena, Juana; Supply, Philip; Locht, Camille
SO PCT Int. Appl., 55 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9807847	A1	19980226	WO 1997-FR1483	19970812

W: AU, CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
FR 2752425 A1 19980220 FR 1996-10277 19960819
FR 2752425 B1 19981113
AU 9740183 A1 19980306 AU 1997-40183 19970812
EP 918854 A1 19990602 EP 1997-937621 19970812
R: DE, ES, FR, GB, IT
JP 2000516471 T2 20001212 JP 1998-510460 19970812
PRAI FR 1996-10277 A 19960819
WO 1997-FR1483 W 19970812
AB Sequences that are specific to members of the ***Mycobacterium*** tuberculosis complex and that can differentiate them from ***Mycobacterium*** BCG are described for use in the diagnosis of infection. These sequences are from the intercistronic region between two genes (senX3 and regX3) for a membrane-bound signal transduction system, rather than from the more frequently used IS6110. The region has an internal deletion in members of the M. tuberculosis group and a pair of probes for the longer and shorter sequences can distinguish M. tuberculosis from ***Mycobacterium*** BCG. The length polymorphism can be detected by PCR or by hybridization.

L4 ANSWER 16 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 11
AN 1998:497675 BIOSIS
DN PREV199800497675
TI Molecular characterization of the ***mycobacterial*** heparin-binding hemagglutinin, a ***mycobacterial*** adhesin.
AU Menozzi, Franco D.; Bischoff, Rainer; Fort, Emmanuelle; Brennan, Michael J.; ***Locht, Camile (1)***
CS (1) Lab. Microbiol., Genet. Mol., Inst. Natl. Sante Recherche Med. U447, Inst. Pasteur Lille, 1 rue Calmette, F-59019 Lille Cedex France
SO Proceedings of the National Academy of Sciences of the United States of America, (Oct. 13, 1998) Vol. 95, No. 21, pp. 12625-12630. ISSN: 0027-8424.
DT Article
LA English
AB Although it generally is accepted that the interaction of ***Mycobacterium*** tuberculosis with alveolar macrophages is a key step in the pathogenesis of tuberculosis, interactions with other cell types, especially epithelia(cells, also may be important. In this study we describe the molecular characterization of a ***mycobacterial*** heparin-binding hemagglutinin (HBHA), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from M. tuberculosis and bacillus Calmette-Guerin, and the sequence was found to be identical between the two species. The calculated M, was smaller than the observed Mr when analyzed by SDS/PAGE. This difference can be attributed to the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of HBHA appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of HBHA to sulfated carbohydrates. Evidence suggests that glycosylation is also important for HBHA-mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of HBHA raises the possibility that this protein is not secreted via the general secretion pathway.

L4 ANSWER 17 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 12
AN 1999:36483 BIOSIS
DN PREV199900036483
TI Systemic and mucosal immune responses after intranasal administration of recombinant ***Mycobacterium*** bovis bacillus calmette-guerin expressing glutathione S-transferase from Schistosoma haematobium.
AU Kremer, Laurent; Dupre, Loic; Riveau, Gilles; Capron, Andre; ***Locht,*** *** Camille (1)***
CS (1) Lab. Microbiol. Genet. Mol., INSERM U447, Inst. Pasteur de Lille, 1 rue du Professeur Calmette, F-59019 Lille Cedex France
SO Infection and Immunity, (Dec., 1998) Vol. 66, No. 12, pp. 5669-5676.
ISSN: 0019-9567.
DT Article
LA English
AB A major goal of current vaccine development is the induction of strong immune responses against protective antigens delivered by mucosal routes. One of the most promising approaches in that respect relies on the use of live recombinant vaccine carriers. In this study, ***Mycobacterium*** bovis BCG was engineered to produce an intracellular glutathione S-transferase from Schistosoma haematobium (Sh28GST). The gene encoding Sh28GST was placed under the control of the ***mycobacterial*** hsp60 promoter on a replicative shuttle plasmid containing a mercury resistance operon as the only selectable marker. The recombinant Sh28GST produced in BCG bound glutathione and expressed enzymatic activity, indicating that its active site was properly folded. Both intraperitoneal and intranasal immunizations of BALB/c mice with the recombinant BCG resulted in strong anti-Sh28GST antibody responses, which were enhanced by a boost. Mice immunized intranasally produced a mixed response with the production of Sh28GST-specific immunoglobulin G1 (IgG1), IgG2a, IgG2b, and IgA in the serum. In addition, high levels of anti-Sh28GST IgA were also found in the bronchoalveolar lavage fluids, demonstrating that intranasal delivery of the recombinant BCG was able to induce long-lasting secretory and systemic immune responses to antigens expressed intracellularly. Surprisingly, intranasal immunization with the BCG producing the Sh28GST induced a much stronger specific humoral response than intranasal immunization with BCG producing the glutathione S-transferase from Schistosoma mansoni, although the two antigens have over 90% identity. This difference was not observed after intraperitoneal administration.

L4 ANSWER 18 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 13
AN 1998:432797 BIOSIS
DN PREV199800432797
TI Specific differentiation between ***Mycobacterium*** bovis BCG and virulent strains of the ***Mycobacterium*** tuberculosis complex.
AU Magdalena, Juana; Supply, Philip; ***Locht, Camille (1)***
CS (1) INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette, F-59019 Lille Cedex France
SO Journal of Clinical Microbiology, (Sept., 1998) Vol. 36, No. 9, pp. 2471-2476.
ISSN: 0095-1137.
DT Article
LA English
AB A PCR procedure based on the intergenic region (IR) separating two genes encoding a recently identified ***mycobacterial*** two-component system, named SenX3-RegX3, was developed and was shown to be suitable for

identifying ***Mycobacterium*** bovis BCG. The senX3-regX3 IR contains a novel type of repetitive sequence, called ***mycobacterial*** interspersed repetitive units (MIRUs). All tested BCG strains exclusively contained 77-bp MIRUs within the senX3-regX3 IR, whereas all non-BCG M. tuberculosis complex strains contained a 53-bp MIRU, in addition to the 77-bp MIRUs. All 148 strains analyzed so far could be divided into eight different groups according to the copy numbers of the 77-bp MIRU and to the presence or absence of the 53-bp MIRU. BCG strains contained either one, two, or three 77-bp MIRUs. The other strains contained one to five 77-bp MIRUs invariably followed by a 53-bp MIRU. The consistent absence of the 53-bp MIRU in BCG strains and its presence in virulent strains allowed us to develop an enzyme-linked immunosorbent assay using specific capture oligonucleotide probes to distinguish between BCG and other M. tuberculosis complex strains.

L4 ANSWER 19 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 14
AN 1998:227261 BIOSIS
DN PREV199800227261
TI Identification of a new DNA region specific for members of ***Mycobacterium*** tuberculosis complex.
AU Magdalena, Juana; Vachee, Anne; Supply, Philip; ***Locht, Camille (1)***
CS (1) INSERM U447, Inst. Pasteur de Lille, 1 rue Prof. Calmette, F-59019 Lille Cedex France
SO Journal of Clinical Microbiology, (April, 1998) Vol. 36, No. 4, pp. 937-943.
ISSN: 0095-1137.
DT Article
LA English
AB The successful use of DNA amplification for the detection of tuberculous ***mycobacteria*** crucially depends on the choice of the target sequence, which ideally should be present in all tuberculous ***mycobacteria*** and absent from all other bacteria. In the present study we developed a PCR procedure based on the intergenic region (IR) separating two genes encoding a recently identified ***mycobacterial*** two-component system named SenX3-RegX3. The senX3-regX3 IR is composed of a novel type of repetitive sequence, called ***mycobacterial*** interspersed repetitive units (MIRUs). In a survey of 116 ***Mycobacterium*** tuberculosis strains characterized by different IS6110 restriction fragment length polymorphisms, 2 ***Mycobacterium*** africanum strains, 3 ***Mycobacterium*** bovis strains (including 2 BCG strains), and 1 ***Mycobacterium*** microti strain, a specific PCR fragment was amplified in all cases. This collection included M. tuberculosis strains that lack IS6110 or mtp40, two target sequences that have previously been used for the detection of M. tuberculosis. No PCR fragment was amplified when DNA from other organisms was used, giving a sensitivity of 100% and a specificity of 100% in the confidence limit of this study. The numbers of MIRUs were found to vary among strains, resulting in six different groups of strains on the basis of the size of the amplified PCR fragment. However, the vast majority of the strains (approximately 90%) fell within the same group, containing two 77-bp MIRUs followed by one 53-bp MIRU.

L4 ANSWER 20 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 15
AN 1999:60457 BIOSIS
DN PREV199900060457

TI Green fluorescent protein as a reporter in rapid screening of antituberculosis compounds in vitro and in macrophages.
AU Srivastava, Ranjana; Deb, Dilip K.; Srivastava, K. K.; ***Locht,*** *** Camille*** ; Srivastava, Brahm S. (1)
CS (1) Div. Microbiol., Central Drug Res. Inst., Lucknow 226001 India
SO Biochemical and Biophysical Research Communications, (Dec. 18, 1998) Vol. 253, No. 2, pp. 431-436.
ISSN: 0006-291X.
DT Article
LA English
AB The development of new drugs against ***Mycobacterium*** tuberculosis is impeded by slow growth and highly infectious nature of the organism that warrants the need to work under highly stringent biosafety conditions. These problems can be overcome by use of reporter genes and surrogate strains. A strain of rapidly growing M. aurum has been recommended as test organism to screen inhibitors of ***mycobacteria*** to preselect compounds for progression into testing against M. tuberculosis. We have investigated the application of recombinant M. aurum expressing green fluorescent protein. in rapid screening of antituberculosis compounds in vitro and in infected macrophages. Recombinant M. aurum(pGFM-11) expressing green fluorescent protein was constructed. The assay is based on measurement of fluorescent intensity at 509 nm. A good correlation was found between fluorescence and growth. Fluorescence of recombinant M. aurum was inhibited in vitro within 8 to 24 h by frontline antimycobacterial drugs at their reported MICs whereas inhibition in infected macrophages was observed in 72 h. Therefore green fluorescent reporter system provides a convenient screen to test antimycobacterial compounds that are active in vitro and within infected macrophages.

L4 ANSWER 21 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1997:776266 CAPLUS
DN 128:58314
TI Cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection
IN Menozzi, Franco; ***Locht, Camille***
PA Institut Pasteur De Lille, Fr.; Institut National De La Sante Et De La Recherche Medicale; Menozzi, Franco; Locht, Camille
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9744463	A2	19971127	WO 1997-FR886	19970520
WO 9744463	A3	19980129		
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2748748	A1	19971121	FR 1996-6168	19960517
FR 2748748	B1	19981106		
AU 9730362	A1	19971209	AU 1997-30362	19970520
EP 914437	A2	19990512	EP 1997-925109	19970520
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, FI
PRAI FR 1996-6168 19960517
WO 1997-FR886 19970520
AB The invention features proteins enabling ***mycobacteria*** adhesion to host cells, in particular to epithelial cells. More particularly, the invention features a ***mycobacterial*** antigen of the heparin-binding hemagglutinin type (HBHA) obtained from ***Mycobacterium*** bovis BCG or M. tuberculosis. The invention particularly features the expression product of an Escherichia coli strain transformed with an HBHA gene. These protein sequences can be used in immunogenic compns., for prepg. vaccines against ***mycobacterial*** infections, and for diagnosis of ***mycobacterial*** infection. Addnl., use of sulfated carbohydrates (such as heparin, chondroitin sulfate, dextran sulfate) to prevent adhesion of ***mycobacteria*** to epithelial cells is claimed. Sera of tuberculosis patients contain antibodies to HBHA while sera of healthy people do not. The post-translational modification of native HBHA (presumably glycosylation) was shown to provide a major antigenic determinant of HBHA.

L4 ANSWER 22 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1998:88157 CAPLUS
DN 128:99777
TI A heparin-binding hemagglutinin of ***Mycobacterium*** for use as an antigen and the gene encoding it
IN Menozzi, Franco; ***Locht, Camille***
PA Institut Pasteur De Lille, Fr.; Institut National de la Sante et de la Recherche Medicale (INSERM)
SO Fr. Demande, 50 pp.
CODEN: FRXXBL
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI FR 2748749	A1	19971121	FR 1996-6169	19960517

AB An adhesion protein of ***Mycobacterium*** responsible for binding to epithelial cells of a host is identified and the gene encoding it is cloned. The protein may be of use as an antigen, or as a target for adhesion-inhibiting drugs. Binding of ***Mycobacterium*** to epithelial cells was inhibited by sulfated polysaccharides such as heparin and chondroitin sulfate, but not by sugars such as galactose. A 28 kDa heparin-binding protein was purified from ***Mycobacterium*** bovis BCG by affinity chromatog. against heparin-Sepharose. The gene was cloned by PCR.

L4 ANSWER 23 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 16
AN 1997:502430 BIOSIS
DN PREV199799801633
TI ***Mycobacterium*** bovis Bacillus Calmette Guerin infection prevents apoptosis of resting human monocytes.
AU Kremer, Laurent; Estaquier, Jerome; Brandt, Eric; Ameisen, Jean-Claude; ***Locht, Camille (1)***
CS (1) Laboratoire de Microbiologie Genetique et Moleculaire INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette, F-59019 Lille Cedex France

SO European Journal of Immunology, (1997) Vol. 27, No. 9, pp. 2450-2456. ISSN: 0014-2980.
DT Article
LA English
AB Apoptosis plays an essential rote in the development and homeostasis of multicellular organisms. Some infectious agents interfere with this programmed cell death to their own benefit. Here, we show that infection of resting human monocytes with ***Mycobacterium*** bovis Bacillus Calmette Guerin (BCG) increases monocyte viability by preventing them from undergoing apoptosis. Heat-killed BCG also prevented apoptosis, indicating that replication of BCG is not required to prevent cell death. Analysis of BCG-infected monocytes revealed an up-regulation of the A1 mRNA, whereas the bcl-2 mRNA was not up-regulated. Interestingly, preinfection with BCG renders the cells resistant to interleukin (IL)-10-induced apoptosis which may be one of the mechanisms ***mycobacteria*** use to modulate immune responses. BCG infection was also accompanied by an impairment of the capacity of monocytes to secrete IL-10 and by an induction of the capacity to secrete tumor necrosis factor-alpha, two cytokines known to induce and prevent human monocyte apoptosis, respectively. Since it has been reported that apoptosis is involved in killing of intracellular ***mycobacteria*** , the prevention of apoptosis may represent a strategy for ***mycobacterial*** survival in the infected host.

L4 ANSWER 24 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 17
AN 1998:46945 BIOSIS
DN PREV199800046945
TI Identification of novel intergenic repetitive units in a ***mycobacterial*** two-component system operon.
AU Supply, Philip; Magdalena, Juana; Himpens, Sabine; ***Locht, Camille*** *** (1)***
CS (1) Lab. Microbiol. Genet. Mol., INSERM U447, Inst. Pasteur Lille, 1, rue Prof. Calmette, F-59019 Lille Cedex France
SO Molecular Microbiology, (Dec., 1997) Vol. 26, No. 5, pp. 991-1003. ISSN: 0950-382X.
DT Article
LA English
AB ***Mycobacterial*** interspersed repetitive units (MIRUs), a novel class of repeated sequences, were identified within the intercistronic region of an operon coding for a ***mycobacterial*** two-component system, named senX3-regX3. Southern blot analysis and homology searches revealed the presence of several homologous sequences in intergenic regions dispersed throughout the genomes of ***Mycobacterium*** bovis BCG, ***Mycobacterium*** tuberculosis and ***Mycobacterium*** leprae. These could be grouped into three major families, containing elements of 77-101 bp, 46-53 bp and 58-101 bp. Based on the available ***mycobacterial*** sequences, the total number of MIRUs is estimated to be about 40-50 per genome. Similar to previously identified small repetitive sequences, the MIRUs of the two-component operon are transcribed on a polycistronic mRNA. Unlike previously identified small repetitive sequences, however, MIRUs do not contain dyad symmetries, comprise small open reading frames (ORFs) whose extremities overlap those of the contiguous ORFs and are oriented in the same translational direction as those of the adjacent genes. Analyses of the sequences at the insertion sites suggest that MIRUs disseminate by transposition into DTGA sites involved in translational coupling in polycistronic operons.

L4 ANSWER 25 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 18
AN 1996:317080 BIOSIS
DN PREV199699039436
TI Neutralizing antibody responses elicited in mice immunized with recombinant bacillus Calmette-Guerin producing the Schistosoma mansoni glutathione S-transferase.
AU Kremer, Laurent; Riveau, Gillles; Baulard, Alain; Capron, Andre; ***Locht, Camill (1)***
CS (1) Lab. Microbiol. Genet. Mol., INSERM CJF9109, Inst. Pasteur de Lille, 1 rue du Professeur Calmette, F-59019 Lille Cedex France
SO Journal of Immunology, (1996) Vol. 156, No. 11, pp. 4309-4317. ISSN: 0022-1767.
DT Article
LA English
AB Schistosomiasis is a group of severe parasitic diseases, in humans and domestic animals, that are especially of importance in the developing world. No efficacious vaccine is currently available. However, Ab-mediated immune responses against the 28-kDa glutathione S-transferase of Schistosoma mansoni (Sm28GST) appear to be involved in protection. This Ag was produced in recombinant ***Mycobacterium*** bovis bacillus Calmette-Guerin (BCG). The recombinant protein bound glutathione and expressed enzymatic activity, indicating that the active site of Sm28GST was folded properly. Single i.v., i.p., s.c., or intranasal immunizations with rBCG in BALB/c mice resulted in significant anti-Sm28GST Ab responses, which were enhanced by a booster dose. The Ab responses remained high for at least 1 yr after immunization. Analyses of the isotype profiles indicated that i.v. immunized mice produced high titers of anti-Sm28GST IgG2a, and less IgG2b and IgG1. Mice immunized by the s.c. route initially also produced high levels of IgG2a and low titers of IgG1 and IgG2b, but the titers of the latter two isotypes increased gradually thereafter, tending toward a mixed profile. Intraperitoneal immunization provided a mixed profile directly after the first administration. High titers of anti-Sm28GST Abs also corresponded to high levels of neutralization of the enzymatic activity. These results indicate that rBCG induces strong IgG1, IgG2a, and IgG2b, and neutralizing Ab responses against Sm28GST, which has been found to correlate with protection against S. mansoni in humans.

L4 ANSWER 26 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 19
AN 1996:331970 BIOSIS
DN PREV199699054326
TI Efficient homologous recombination in fast-growing and slow-growing ***mycobacteria*** .
AU Baulard, Alain; Kremer, Laurent; ***Locht, Camille (1)***
CS (1) Lab. Microbiol. Genet. Mol., INSERM CJF9109, Inst. Pasteur Lille, 1, Rue Prof. Calmette, F-59019 Lille Cedex France
SO Journal of Bacteriology, (1996) Vol. 178, No. 11, pp. 3091-3098. ISSN: 0021-9193.
DT Article
LA English
AB Although homologous recombination is a major mechanism for DNA rearrangement in most living organisms, it has been difficult to detect in slowly growing ***mycobacteria*** by a classical suicide vector approach. Among the possible reasons for this are the low levels of

transformation efficiency, the relatively high levels of illegitimate recombination, and the peculiar nature of the recA gene in slowly growing ***mycobacteria*** . In this report, we present an efficient homologous recombination system for these organisms based on the use of replicative plasmids which facilitates the detection of rare recombination events, because the proportions of recombined molecules increase over time. Intraplasmid homologous recombination in ***Mycobacterium*** smegmatis and ***Mycobacterium*** bovis BCG was easily selected by the reconstitution of an interrupted kanainycin resistance gene. Chromosomal integration via homologous recombination was selected by the expression of the kanamycin resistance gene under the control of a chromosomal promoter that was not present in the plasmid before recombination. This technique was termed STORE (for selection technique of recombination events). All the clones selected by STORE had undergone homologous recombination, as evidenced by PCR analyses of the kanamycin-resistant clones. This technique should be applicable to all organisms for which homologous recombination has been difficult to achieve, provided the gene of interest is expressed.

L4 ANSWER 27 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 20
AN 1996:510320 BIOSIS
DN PREV199699232676
TI Identification of a heparin-binding hemagglutinin present in ***mycobacteria*** .
AU Menozzi, Franco D.; Rouse, Julie H.; Alavi, Mohammad; Laude-Sharp, Marilyn; Muller, Jacqueline; Bischoff, Rainer; Brennan, Michael J. (1); ***Locht, Camille***
CS (1) CBER/FDA, 1401 Rockville Pike (HFM-431), Rockville, MD 20852-1448 USA
SO Journal of Experimental Medicine, (1996) Vol. 184, No. 3, pp. 993-1001. ISSN: 0022-1007.
DT Article
LA English
AB Adherence to mammalian host tissues is an important virulence trait in microbial pathogenesis, yet little is known about the adherence mechanisms of ***mycobacteria*** . Here, we show that binding of ***mycobacteria*** to epithelial cells but not to macrophages can be specifically inhibited by sulfated carbohydrates. Using heparin-Sepharose chromatography, a 28-kD heparin-binding protein was purified from culture supernatants and cell extracts of ***Mycobacterium*** bovis and ***Mycobacterium*** tuberculosis. This protein, designated heparin-binding hemagglutinin (HBHA), promotes the agglutination of rabbit erythrocytes, which is specifically inhibited by sulfated carbohydrates. HBHA also induces ***mycobacterial*** aggregation, suggesting that it can mediate bacteria-bacteria interactions as well. Hemagglutination, ***mycobacterial*** aggregation, as well as attachment to epithelial cells are specifically inhibited in the presence of anti-HBHA antibodies. Immunoelectron microscopy using anti-HBHA monoclonal antibodies revealed that the protein is surface exposed, consistent with a role in adherence. Immunoblot analyses using antigen-specific antibodies indicated that HBHA is different from the fibronectin-binding proteins of the antigen 85 complex and p55, and comparison of the NH-2-terminal amino acid sequence of purified HBHA with the protein sequence data bases did not reveal any significant similarity with other known proteins. Sera from tuberculosis patients but not from healthy individuals were found to recognize HBHA, indicating its immunogenicity in humans during ***mycobacterial***

infections. Identification of putative ***mycobacterial*** adhesins, such as the one described in this report, may provide the basis for the development of new therapeutic and prophylactic strategies against ***mycobacterial*** diseases.

L4 ANSWER 28 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 21
AN 1996:575516 BIOSIS
DN PREV199799290197
TI A new series of ***mycobacterial*** expression vectors for the development of live recombinant vaccines.
AU Baulard, Alain; Kremer, Laurent; Supply, Philip; Vidaud, Dominique; Bidart, Jean-Michel; Bellet, Dominique; ***Locht, Camille (1)***
CS (1) Lab. Microbiol. Genet. Mol., INSERM CJF9109, Inst. Pasteur de Lille, 1 rue du Prof. Calmette, F-59019 Lille Cedex France
SO Gene (Amsterdam), (1996) Vol. 176, No. 1-2, pp. 149-154. ISSN: 0378-1119.
DT Article
LA English
AB Recombinant BCG (bacillus Calmette-Guerin) is a promising candidate as a live vaccine delivery system. Thus far, however, only autoreplicative plasmids carrying the heterologous genes to be expressed in BCG, together with antibiotic-resistance genes, have been successfully used. This could potentially lead to the spreading of antibiotic resistance among other bacteria, and might therefore be unsafe for the environment. In this study, we present a series of three Escherichia coli- ***Mycobacteria*** shuttle vectors which enable expression and secretion of antigens without the use of antibiotic-resistance markers. All these plasmids confer mercury resistance to the host bacteria as the only selectable marker and contain a unique restriction site to allow for single-step in-frame cloning of open reading frames downstream from the ***Mycobacterium*** tuberculosis 85A antigen promoter and export signal. The system was used to express the free beta-subunit of human chorionic gonadotropin (hCG-beta), a potential target of an immunotherapeutic vaccine.

L4 ANSWER 29 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1996:76580 CAPLUS
DN 124:108958
TI Shuttle vectors for use with ***Mycobacteria*** and the use of transgenic ***Mycobacteria*** in vaccines
IN Escuyer, Vincent; Baulard, Alain; Berche, Patrick; ***Locht, Camille*** ; Haddad, Nadia
PA Institut National de la Sante et de la Recherche Medicale (INSERM), Fr.; Institut Pasteur; Institut Pasteur de Lille
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9532296	A1	19951130	WO 1995-FR664	19950519
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2720076	A1	19951124	FR 1994-6202	19940520
FR 2720076	B1	19960802		

CA 2190861 AA 19951130 CA 1995-2190861 19950519
AU 9526203 A1 19951218 AU 1995-26203 19950519
EP 760859 A1 19970312 EP 1995-920967 19950519
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
JP 10503926 T2 19980414 JP 1995-530099 19950519
US 6074866 A 20000613 US 1997-737588 19970212
PRAI FR 1994-6202 A 19940520
WO 1995-FR664 W 19950519
AB Shuttle vectors for the introduction of DNA into ***Mycobacteria*** have replications origins functional in the ***mycobacterial*** host and in a more convenient cloning host and a no. of convenient cloning sites. The vector also carries a heavy metal resistance marker for selection of transformants. The preferred resistance marker is the merA gene or mercury resistance operon of Tn501 although an arsenic resistance marker may also be used. Transgenic ***mycobacteria*** expressing the gene for a foreign antigen may be used in vaccines. Two vectors, pMR001 and pVN2, using mercury and kanamycin resistance markers were constructed in Escherichia coli and ***Mycobacterium*** transferred with them showed increased resistance to mercury. A plasmid using the ars gene conferred resistance to 10 mg/mL AsNO3.

L4 ANSWER 30 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 22
AN 1995:297833 BIOSIS
DN PREV199598312133
TI Mercury resistance as a selective marker for recombinant ***mycobacteria*** .
AU Baulard, Alain; Escuyer, Vincent; Haddad, Nadia; Kremer, Laurent; ***Locht, Camille (1)*** ; Berche, Patrick
CS (1) Lab. Microbiol. Genetique Mol. INSERM CJF9109, Inst. Pasteur de Lille, 1 Rue du Prof. Calmette, F-59019 Lille Cedex France
SO Microbiology (Reading), (1995) Vol. 141, No. 4, pp. 1045-1050. ISSN: 1350-0872.
DT Article
LA English
AB The use of antibiotic-resistance markers for the selection of recombinant ***mycobacteria*** is widespread but questionable considering the development of live recombinant BCG vaccines. In contrast, vector-encoded resistance to heavy metals such as mercury may represent an interesting alternative for the development of live vaccines compatible with use in humans and in animals. The mercury resistance genes (mer) from Pseudomonas aeruginosa and from Serratia marcescens were cloned into the Escherichia coli- ***Mycobacterium*** shuttle vector pRR3. The resulting vectors, designated pMR001 and pVN2, were introduced by electroporation into ***Mycobacterium*** smegmatis, ***Mycobacterium*** bovis BCG and ***Mycobacterium*** tuberculosis. The recombinant ***mycobacteria*** were stable in vitro and in vivo, and had high-level mercury resistance, thus indicating that the mer genes can be useful as selective markers in ***mycobacteria*** .

L4 ANSWER 31 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 23
AN 1995:547839 BIOSIS
DN PREV199698562139
TI Green fluorescent protein as a new expression marker in ***mycobacteria*** .
AU Kremer, Laurent; Baulard, Alain; Estaquier, Jerome; Poulain-Godefroy,

Odile; ***Locht, Camille (1)***

CS (1) Lab. Microbiol. Genetique Moleculaire, INSERM CJF9109, Inst. Pasteur de Lille, 1 rue du Professeur Calmette, F-59019 Lille Cedex France

SO Molecular Microbiology, (1995) Vol. 17, No. 5, pp. 913-922. ISSN: 0950-382X.

DT Article

LA English

AB This study describes the use and the advantages of the green fluorescent protein (GFP) as a reporter molecule for ***mycobacteria*** . The gfp gene from Aequorea victoria was placed under the control of the hsp60 promoter in the shuttle vector pGFM-11. The gfp expression in the recombinant ***Mycobacterium*** smegmatis and BCG was readily detected on agar plates by the development of an intense green fluorescence upon irradiation with long-wave u.v. light. In ***mycobacteria*** containing a pGFM-11 derivative that lacks the hsp60 promoter, no fluorescence was observed. However, this plasmid was successfully used as a promoter-probe vector to identify BCG promoters. The fluorescence emission of GFP in ***mycobacteria*** harbouring pGFM-11 and grown in liquid media could be quantified by spectrofluorimetry. This allowed for easy assessment of drug susceptibility. As GFP does not require the addition of substrates or co-factors, the green fluorescent bacilli could be directly observed within infected macrophages using fluorescence and laser confocal microscopy, or in tissue sections of infected mice. Finally, infected cells or free-living recombinant ***mycobacteria*** could also be analysed by flow cytometry. The GFP thus appears to be a convenient reporter for ***mycobacteria*** , allowing tracing of recombinant ***mycobacteria*** , isolation of promoters with interesting properties, in vivo drug testing and the development of new diagnostic tools.

L4 ANSWER 32 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 24

AN 1995:124295 BIOSIS

DN PREV199598138595

TI Analysis of the ***Mycobacterium*** tuberculosis 85A Antigen Promoter Region.

AU Kremer, Laurent; Baulard, Alain; Estaquier, Jerome; Content, Jean; Capron, Andre; ***Locht, Camille (1)***

CS (1) Lab. Microbiol. Genet. Mol. INSERM CJF9109, 1, rue Prof. Calmette, F-59019 Lille Cedex France

SO Journal of Bacteriology, (1995) Vol. 177, No. 3, pp. 642-653. ISSN: 0021-9193.

DT Article

LA English

AB A ***mycobacterial*** expression-secretion vector was constructed in which the Escherichia coli alkaline phosphatase (phoA) reporter gene was placed under the control of the ***Mycobacterium*** tuberculosis 85A promoter and secretion signal sequences. In recombinant ***Mycobacterium*** smegmatis and ***Mycobacterium*** bovis BCG, PhoA activity could readily be detected on the ***mycobacterial*** cell surface and in the culture supernatant, indicating that the 85A signals can drive heterologous expression and secretion in both species. In contrast to the ***mycobacteria*** , the 85A promoter did not function in E. coli. We mapped the promoter region by progressive deletions using BAL 31 exonuclease and by primer extension analysis. Insertion and deletion mutations within the promoter region indicated

that, unlike most E. coli promoters but similar to Streptomyces promoters, the position of the putative -35 region was not critical for efficient promoter activity. In addition, we investigated the ability of the identified signals to drive the production and secretion in BCG of recombinant Schistosoma masoni glutathione S-transferase (Sm28GST), a protective antigen against schistosomiasis. BALB/c mice immunized with the recombinant BCG by a single dose exhibited a weak but specific T-cell response to Sm28GST.

L4 ANSWER 33 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1995:147968 BIOSIS
DN PREV199598162268
TI Cloning of a complete two-component regulatory system of ***Mycobacterium*** bovis BCG.
AU Supply, Philip; ***Locht, Camille***
CS Lab. Microbiol. Genet. Mol., Inst. Pasteur de Lille, 1 Rue du Professeur Calmette, F-59019 Lille France
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 79.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis Tamarron, Colorado, USA February 19-25, 1995
ISSN: 0733-1959.
DT Conference
LA English

L4 ANSWER 34 OF 34 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1995:147915 BIOSIS
DN PREV199598162215
TI Identification of a heparin-binding ***mycobacterial*** hemagglutinin.
AU Brennan, Michael J. (1); Laude-Sharp, Maryline (1); Hannah, Julie H. (1); Menozzi, Franco; ***Locht, Camille***
CS (1) DBP, CBER, FDA, Bethesda, MD 20852 USA
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 66.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis Tamarron, Colorado, USA February 19-25, 1995
ISSN: 0733-1959.
DT Conference
LA English

=> s hbha and mycobact?
L5 48 HBHA AND MYCOBACT?

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 11 DUP REM L5 (37 DUPLICATES REMOVED)

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YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
AN 2001:867419 CAPLUS
TI ***Mycobacterial*** protein ***HbhA*** binds human complement

component C3
AU Mueller-Ortiz, Stacey L.; Wanger, Audrey R.; Norris, Steven J.
CS Graduate School of Biomedical Sciences and Department of Pathology and Laboratory Medicine, Medical School, University of Texas Health Science Center at Houston, Houston, TX, 77030, USA
SO Infect. Immun. (2001), 69(12), 7501-7511
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB ***Mycobacterium*** tuberculosis and ***Mycobacterium*** avium are facultative intracellular pathogens that are able to survive and replicate in mononuclear phagocytes. Human complement component C3 has previously been shown to mediate attachment and phagocytosis of these bacteria by mononuclear phagocytes. In this study, a C3 ligand affinity blot protocol was used to identify a 30-kDa C3-binding protein in M. tuberculosis and ***Mycobacterium*** smegmatis and a 31-kDa C3-binding protein in M. avium. The C3-binding proteins in M. tuberculosis and M. avium localized to the cell membrane fraction and partitioned to the detergent fraction during Triton X-114 phase partitioning. The C3-binding protein from M. tuberculosis was partially purified using a cation exchange column and was shown to bind Con A. The N terminus and an internal fragment of the partially purified C3-binding protein were subjected to amino acid sequence anal. The resulting amino acid sequences matched the M. tuberculosis heparin-binding hemagglutinin (***HbhA***) protein. Recombinant full-length ***HbhA*** and the C terminus of ***HbhA*** fused to maltose-binding protein, but not recombinant ***HbhA*** lacking the C-terminal region, bound human C3. Recombinant full-length ***HbhA*** coated on polystyrene beads, was found to enhance the adherence and/or phagocytosis of the coated beads to J774.A1 cells in both the presence and absence of human serum. The presence of complement-sufficient serum increased the adherence of the ***HbhA*** -coated beads to the J774.A1 cells in a C3-dependent manner. If ***HbhA*** within the bacterial cell membrane functions similarly to isolated ***HbhA*** , this protein may enhance the adherence and phagocytosis of M. tuberculosis and M. avium to mononuclear phagocytes through the binding of C3 and interaction with C3 receptors on mononuclear phagocytes.
RE.CNT 47
RE
(1) Armitige, L; Infect Immun 2000, V68, P767 CAPLUS
(2) Arnaout, M; Blood 1990, V75, P1037 CAPLUS
(3) Bellinger-Kawahara, C; J Exp Mod 1990, V172, P1201 CAPLUS
(4) Bennett, B; J Immunol Methods 1992, V153, P31 CAPLUS
(5) Bermudez, L; Infect Immun 1991, V59, P1697 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI ***Mycobacterial*** protein ***HbhA*** binds human complement component C3
AB ***Mycobacterium*** tuberculosis and ***Mycobacterium*** avium are facultative intracellular pathogens that are able to survive and replicate in mononuclear phagocytes. Human complement component C3 has previously been shown to mediate attachment and phagocytosis of these bacteria by mononuclear phagocytes. In this study, a C3 ligand affinity blot protocol was used to identify a 30-kDa C3-binding protein in M. tuberculosis and ***Mycobacterium*** smegmatis and a 31-kDa C3-binding protein in M.

avium. The C3-binding proteins in M. tuberculosis and M. avium localized to the cell membrane fraction and partitioned to the detergent fraction during Triton X-114 phase partitioning. The C3-binding protein from M. tuberculosis was partially purified using a cation exchange column and was shown to bind Con A. The N terminus and an internal fragment of the partially purified C3-binding protein were subjected to amino acid sequence anal. The resulting amino acid sequences matched the M. tuberculosis heparin-binding hemagglutinin (***HbhA***) protein. Recombinant full-length ***HbhA*** and the C terminus of ***HbhA*** fused to maltose-binding protein, but not recombinant ***HbhA*** lacking the C-terminal region, bound human C3. Recombinant full-length ***HbhA*** coated on polystyrene beads, was found to enhance the adherence and/or phagocytosis of the coated beads to J774.A1 cells in both the presence and absence of human serum. The presence of complement-sufficient serum increased the adherence of the ***HbhA*** -coated beads to the J774.A1 cells in a C3-dependent manner. If ***HbhA*** within the bacterial cell membrane functions similarly to isolated ***HbhA*** , this protein may enhance the adherence and phagocytosis of M. tuberculosis and M. avium to mononuclear phagocytes through the binding of C3 and interaction with C3 receptors on mononuclear phagocytes.

L6 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 2
AN 2001:528989 BIOSIS
DN PREV200100528989
TI The heparin-binding haemagglutinin of M. tuberculosis is required for extrapulmonary dissemination.
AU Pethe, Kevin; Alonso, Sylvie; Biet, Franck; Delogu, Giovanni; Brennan, Michael J.; Locht, Camille (1); Menozzi, Franco D.
CS (1) Unite INSERM U447, Institut Pasteur de Lille, 1 rue du Prof. Calmette, F-59019, Lille Cedex: camille.locht@pasteur.lille.fr France
SO Nature (London), (12 July, 2001) Vol. 412, No. 6843, pp. 190-194. print. ISSN: 0028-0836.
DT Article
LA English
SL English
AB Tuberculosis remains the world's leading cause of death due to a single infectious agent, ***Mycobacterium*** tuberculosis, with 3 million deaths and 10 million new cases per year. The infection initiates in the lungs and can then spread rapidly to other tissues. The availability of the entire M. tuberculosis genome sequence and advances in gene disruption technologies have led to the identification of several ***mycobacterial*** determinants involved in virulence. However, no virulence factor specifically involved in the extrapulmonary dissemination of M. tuberculosis has been identified to date. Here we show that the disruption of the M. tuberculosis or ***Mycobacterium*** bovis Bacille Calmette-Guerin (BCG) ***hbhA*** gene encoding the heparin-binding haemagglutinin adhesin (***HBHA***) markedly affects ***mycobacterial*** interactions with epithelial cells, but not with macrophage-like cells. When nasally administered to mice, the mutant strains were severely impaired in spleen colonization, but not in lung colonization. Coating wild-type ***mycobacteria*** with anti-***HBHA*** antibodies also impaired dissemination after intranasal infection. These results provide evidence that adhesins such as ***HBHA*** are required for extrapulmonary dissemination, and that

interactions with non-phagocytic cells have an important role in the pathogenesis of tuberculosis. They also suggest that antibody responses to ***HBHA*** may add to immune protection against tuberculosis.

AB Tuberculosis remains the world's leading cause of death due to a single infectious agent, ***Mycobacterium*** tuberculosis, with 3 million deaths and 10 million new cases per year. The infection initiates in the lungs and can. . . of the entire M. tuberculosis genome sequence and advances in gene disruption technologies have led to the identification of several ***mycobacterial*** determinants involved in virulence. However, no virulence factor specifically involved in the extrapulmonary dissemination of M. tuberculosis has been identified to date. Here we show that the disruption of the M. tuberculosis or ***Mycobacterium*** bovis Bacille Calmette-Guerin (BCG) ***hbhA*** gene encoding the heparin-binding haemagglutinin adhesin (***HBHA***) markedly affects ***mycobacterial*** interactions with epithelial cells, but not with macrophage-like cells. When nasally administered to mice, the mutant strains were severely impaired in spleen colonization, but not in lung colonization. Coating wild-type ***mycobacteria*** with anti-***HBHA*** antibodies also impaired dissemination after intranasal infection. These results provide evidence that adhesins such as ***HBHA*** are required for extrapulmonary dissemination, and that interactions with non-phagocytic cells have an important role in the pathogenesis of tuberculosis. They also suggest that antibody responses to ***HBHA*** may add to immune protection against tuberculosis.

BC ***Mycobacteriaceae*** 08881
Muridae 86375

ORGN Super Taxa
Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; ***Mycobacteriaceae*** : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Mycobacterium bovis BCG (***Mycobacteriaceae***); ***Mycobacterium*** tuberculosis (***Mycobacteriaceae***): pathogen; mouse (Muridae)

ORGN Organism Superterms
Animals; Bacteria; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

L6 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 3

AN 2001:71458 BIOSIS

DN PREV200100071458

TI ***Mycobacterium*** smegmatis laminin-binding glycoprotein shares epitopes with ***Mycobacterium*** tuberculosis heparin-binding haemagglutinin.

AU Pethe, Kevin; Puech, Virginie; Daffe, Mamadou; Josenhans, Christine; Drobecq, Herve; Locht, Camille; Menozzi, Franco D. (1)

CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, 1 Rue A. Calmette, 59019, Lille Cedex: franco.menozzi@pasteur-lille.fr France

SO Molecular Microbiology, (January, 2001) Vol. 39, No. 1, pp. 89-99. print. ISSN: 0950-382X.

DT Article

LA English

SL English

AB ***Mycobacterium*** tuberculosis, the causative agent of tuberculosis,

produces a heparin-binding haemagglutinin adhesin (***HBHA***), which is involved in its epithelial adherence. To ascertain whether ***HBHA*** is also present in fast-growing ***mycobacteria*** , ***Mycobacterium*** smegmatis was studied using anti- ***HBHA*** monoclonal antibodies (mAbs). A cross-reactive protein was detected by immunoblotting of M. smegmatis whole-cell lysates. However, the M. tuberculosis ***HBHA*** -encoding gene failed to hybridize with M. smegmatis chromosomal DNA in Southern blot analyses. The M. smegmatis protein recognized by the anti- ***HBHA*** mAbs was purified by heparin-Sepharose chromatography, and its amino-terminal sequence was found to be identical to that of the previously described histone-like protein, indicating that M. smegmatis does not produce ***HBHA*** . Biochemical analysis of the M. smegmatis histone-like protein shows that it is glycosylated like ***HBHA*** . Immunoelectron microscopy demonstrated that the M. smegmatis protein is present on the ***mycobacterial*** surface, a cellular localization inconsistent with a histone-like function, but compatible with an adhesin activity. In vitro protein interaction assays showed that this glycoprotein binds to laminin, a major component of basement membranes. Therefore, the protein was called M. smegmatis laminin-binding protein (MS-LBP). MS-LBP does not appear to be involved in adherence in the absence of laminin but is responsible for the laminin-mediated ***mycobacterial*** adherence to human pneumocytes and macrophages. Homologous laminin-binding adhesins are also produced by virulent ***mycobacteria*** such as M. tuberculosis and ***Mycobacterium*** leprae, suggesting that this adherence mechanism may contribute to the pathogenesis of ***mycobacterial*** diseases.

TI ***Mycobacterium*** smegmatis laminin-binding glycoprotein shares epitopes with ***Mycobacterium*** tuberculosis heparin-binding haemagglutinin.

AB ***Mycobacterium*** tuberculosis, the causative agent of tuberculosis, produces a heparin-binding haemagglutinin adhesin (***HBHA***), which is involved in its epithelial adherence. To ascertain whether ***HBHA*** is also present in fast-growing ***mycobacteria*** , ***Mycobacterium*** smegmatis was studied using anti- ***HBHA*** monoclonal antibodies (mAbs). A cross-reactive protein was detected by immunoblotting of M. smegmatis whole-cell lysates. However, the M. tuberculosis ***HBHA*** -encoding gene failed to hybridize with M. smegmatis chromosomal DNA in Southern blot analyses. The M. smegmatis protein recognized by the anti- ***HBHA*** mAbs was purified by heparin-Sepharose chromatography, and its amino-terminal sequence was found to be identical to that of the previously described histone-like protein, indicating that M. smegmatis does not produce ***HBHA*** . Biochemical analysis of the M. smegmatis histone-like protein shows that it is glycosylated like ***HBHA*** . Immunoelectron microscopy demonstrated that the M. smegmatis protein is present on the ***mycobacterial*** surface, a cellular localization inconsistent with a histone-like function, but compatible with an adhesin activity. In vitro protein interaction assays. . . MS-LBP does not appear to be involved in adherence in the absence of laminin but is responsible for the laminin-mediated ***mycobacterial*** adherence to human pneumocytes and macrophages. Homologous laminin-binding adhesins are also produced by virulent ***mycobacteria*** such as M. tuberculosis and ***Mycobacterium*** leprae, suggesting that this adherence mechanism may contribute to the pathogenesis of ***mycobacterial*** diseases.

IT . . .

system; pneumocyte: respiratory system
IT Chemicals & Biochemicals
DNA: chromosomal; anti-heparin-binding hemagglutinin adhesion monoclonal antibodies; cross-reactive protein; heparin-binding hemagglutinin adhesion [***HBHA***]; laminin; laminin-binding glycoprotein
ORGN Super Taxa
Mycobacteriaceae : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Mycobacterium leprae (***Mycobacteriaceae***);
Mycobacterium smegmatis (***Mycobacteriaceae***);
Mycobacterium tuberculosis (***Mycobacteriaceae***)
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L6 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 2000:814638 CAPLUS
DN 133:359789
TI Affinity purification of recombinant proteins
IN Menozzi, Franco; Locht, Camille; Pethe, Kevin
PA Institut Pasteur de Lille, Fr.; Institut National de la Sante et de la Recherche Medicale
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000068398	A1	20001116	WO 2000-FR1282	20000511
FR 2793492	A1	20001117	FR 1999-6031	19990511

PI WO 2000068398 A1 20001116 WO 2000-FR1282 20000511
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
FR 2793492 A1 20001117 FR 1999-6031 19990511
PRAI FR 1999-6031 A 19990511
OS MARPAT 133:359789
AB The invention concerns a novel method for producing and purifying a protein of interest by affinity chromatog. on a solid support bearing sulfated polysaccharides. Thus, the protein is fused at the N- or C-terminus with a label consisting of .gtoreq.1 R1 motifs of formula X1X2Y1Y2PY3 and 0-5 R2 motifs of formula X1X2Y1Y2Y3X3X4 (X1-X4 = Lys or Arg; Y1-Y3 = any amino acid, but preferably Ala, Leu, Ileu, or Val, and P = proline; the no. of R1 and R2 motifs and their combination being selected on the basis of the desired affinity between the polypeptide and the solid support bearing sulfated polysaccharides, provided that when R2 = 0, R1 .gtoreq. 2). Thus, ***Mycobacterium*** tuberculosis heparin-binding hemagglutinin was prepd. with recombinant, protease-deficient E. coli. The hemagglutinin could be affinity purified

using heparin-Sepharose. The affinity of the hemagglutinin for the affinity matrix was dependent on the presence of lysine-rich repeat motifs in the C-terminus of the hemagglutinin. Modifying the no. of these repeat units modified the affinity of the hemagglutinin for the heparin-Sepharose.

RE.CNT 6

RE

(1) Cardin, A; ARTERIOSCLEROSIS V9(1), P21 CAPLUS

(2) Kagaku Oyobi Kessei Ryoho; JP 61054451 A 1986 CAPLUS

(3) Menozzi, F; WO 9744463 A 1997 CAPLUS

(4) Menozzi, F; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1998, V95(21), P12625 CAPLUS

(5) Pasteur Institut; WO 9528486 A 1995 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB The invention concerns a novel method for producing and purifying a protein of interest by affinity chromatog. on a solid support bearing sulfated polysaccharides. Thus, the protein is fused at the N- or C-terminus with a label consisting of .gtoreq.1 R1 motifs of formula X1X2Y1Y2PY3 and 0-5 R2 motifs of formula X1X2Y1Y2Y3X3X4 (X1-X4 = Lys or Arg; Y1-Y3 = any amino acid, but preferably Ala, Leu, Ileu, or Val, and P = proline; the no. of R1 and R2 motifs and their combination being selected on the basis of the desired affinity between the polypeptide and the solid support bearing sulfated polysaccharides, provided that when R2 = 0, R1 .gtoreq. 2). Thus, ***Mycobacterium*** tuberculosis heparin-binding hemagglutinin was prepd. with recombinant, protease-deficient E. coli. The hemagglutinin could be affinity purified using heparin-Sepharose. The affinity of the hemagglutinin for the affinity matrix was dependent on the presence of lysine-rich repeat motifs in the C-terminus of the hemagglutinin. Modifying the no. of these repeat units modified the affinity of the hemagglutinin for the heparin-Sepharose.

ST recombinant fusion protein affinity purifn sulfated polysaccharide; ***Mycobacterium*** hemagglutinin lysine rich repeat purifn heparin agarose

IT Hemagglutinins
RL: BPN (Biosynthetic preparation); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(heparin-binding, of ***Mycobacterium*** tuberculosis; affinity purifn. of recombinant proteins)

IT Plasmid vectors
(pET- ***HBHA*** , ***Mycobacterium*** hemagglutinin expression vector; affinity purifn. of recombinant proteins)

IT Plasmid vectors
(pET-HBHA2C, ***Mycobacterium*** hemagglutinin expression vector; affinity purifn. of recombinant proteins)

L6 ANSWER 5 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 4

AN 2000:345156 BIOSIS

DN PREV200000345156

TI Characterization of the heparin-binding site of the ***mycobacterial*** heparin-binding hemagglutinin adhesin.

AU Pethe, Kevin; Aumercier, Marc; Fort, Emmanuelle; Gatot, Christophe; Locht, Camille (1); Menozzi, Franco D.

CS (1) Mecanismes Moleculaires de la Pathogenie Microbienne, INSERM U447, Institut Pasteur de Lille, Institut de Biologie de Lille, 1 Rue A.

Calmette, 59019, Lille Cedex France
SO Journal of Biological Chemistry, (May 12, 2000) Vol. 275, No. 19, pp. 14273-14280. print.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB The ***mycobacterial*** adhesin heparin-binding hemagglutinin (***HBHA***) contains several lysine-rich repeats at its carboxyl-terminal end. Using truncated recombinant ***HBHA*** forms and hybrid proteins containing ***HBHA*** repeats grafted onto the Escherichia coli maltose-binding protein (MBP), we found that these repeats are responsible for heparin binding. Immunofluorescence microscopy studies revealed that their deletion abrogates binding of ***HBHA*** to human pneumocytes. Conversely, when fused to MBP, the ***HBHA*** repeats confer pneumocyte adherence properties to the hybrid protein. Treatment of pneumocytes with glycosaminoglycan-degrading enzymes showed that ***HBHA*** binding depends on the presence of heparan sulfate chains on the cell surface. The epitope of a monoclonal antibody that inhibits ***mycobacterial*** adherence to epithelial cells was mapped within the lysine-rich repeats, confirming their involvement in ***mycobacterial*** adherence to epithelial cells. Surface plasmon resonance analyses showed that recombinant ***HBHA*** binds to immobilized heparin with fast association kinetics (Ka = 5.62 (+- 0.10) X 105 M-1 s-1), whereas the dissociation kinetics were slower (kd = 0.015 (+- 0.002) s-1), yielding a KD value of 26 nM. Similar analyses with grafted MBP indicated similar kinetic constants, indicating that the carboxyl-terminal repeats contain the entire heparin-binding site of ***HBHA*** . The molecular characterization of the interactions of ***HBHA*** with epithelial glycosaminoglycans should help to better understand ***mycobacterial*** adherence within the lungs and may ultimately lead to new approaches for therapy or immunoprophylaxis.
TI Characterization of the heparin-binding site of the ***mycobacterial*** heparin-binding hemagglutinin adhesin.
AB The ***mycobacterial*** adhesin heparin-binding hemagglutinin (***HBHA***) contains several lysine-rich repeats at its carboxyl-terminal end. Using truncated recombinant ***HBHA*** forms and hybrid proteins containing ***HBHA*** repeats grafted onto the Escherichia coli maltose-binding protein (MBP), we found that these repeats are responsible for heparin binding. Immunofluorescence microscopy studies revealed that their deletion abrogates binding of ***HBHA*** to human pneumocytes. Conversely, when fused to MBP, the ***HBHA*** repeats confer pneumocyte adherence properties to the hybrid protein. Treatment of pneumocytes with glycosaminoglycan-degrading enzymes showed that ***HBHA*** binding depends on the presence of heparan sulfate chains on the cell surface. The epitope of a monoclonal antibody that inhibits ***mycobacterial*** adherence to epithelial cells was mapped within the lysine-rich repeats, confirming their involvement in ***mycobacterial*** adherence to epithelial cells. Surface plasmon resonance analyses showed that recombinant ***HBHA*** binds to immobilized heparin with fast association kinetics (Ka = 5.62 (+- 0.10) X 105 M-1 s-1), whereas the dissociation. . . Similar analyses with grafted MBP indicated similar kinetic constants, indicating that the carboxyl-terminal repeats contain the entire heparin-binding site of ***HBHA*** . The molecular characterization of the interactions of

HBHA with epithelial glycosaminoglycans should help to better understand ***mycobacterial*** adherence within the lungs and may ultimately lead to new approaches for therapy or immunoprophylaxis.

BC ***Mycobacteriaceae*** 08881

ORGN Super Taxa

Mycobacteriaceae : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name

Mycobacterium bovis (***Mycobacteriaceae***)

ORGN Organism Superterms

Bacteria; Eubacteria; Microorganisms

L6 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 5

AN 2000:48801 BIOSIS

DN PREV200000048801

TI Functional domains present in the ***mycobacterial*** hemagglutinin, ***HBHA*** .

AU Delogu, Giovanni; Brennan, Michael J. (1)

CS (1) CBER/FDA, 29 Lincoln Dr. (HFM-431), Bethesda, MD USA

SO Journal of Bacteriology, (Dec., 1999) Vol. 181, No. 24, pp. 7464-7469. ISSN: 0021-9193.

DT Article

LA English

SL English

AB Identification and characterization of ***mycobacterial*** adhesins and complementary host receptors required for colonization and dissemination of ***mycobacteria*** in host tissues are needed for a more complete understanding of the pathogenesis of diseases caused by these bacteria and for the development of effective vaccines. Previous investigations have demonstrated that a 28-kDa heparin-binding ***mycobacterial*** surface protein, ***HBHA*** , can agglutinate erythrocytes and promote ***mycobacterial*** aggregation in vitro. In this study, further molecular and biochemical analysis of ***HBHA*** demonstrates that it has three functional domains: a transmembrane domain of 18 amino acids residing near the N terminus, a large domain of 81 amino acids consistent with an alpha-helical coiled-coil region, and a Lys-Pro-Ala-rich C-terminal domain that mediates binding to proteoglycans. Using His-tagged recombinant ***HBHA*** proteins and nickel chromatography we demonstrate that ***HBHA*** polypeptides which contain the coiled-coil region form multimers. This tendency to oligomerize may be responsible for the induction of ***mycobacterial*** aggregation since a truncated N-terminal ***HBHA*** fragment containing the coiled-coil domain promotes ***mycobacterial*** aggregation. Conversely, a truncated C-terminal ***HBHA*** fragment which contains Lys-Pro-Ala-rich repeats binds to the proteoglycan decorin. These results indicate that ***HBHA*** contains at least three distinct domains which facilitate intercalation into surface membranes, promote bacterium-bacterium interactions, and mediate the attachment to sulfated glycoconjugates found in host tissues.

TI Functional domains present in the ***mycobacterial*** hemagglutinin, ***HBHA*** .

AB Identification and characterization of ***mycobacterial*** adhesins and complementary host receptors required for colonization and dissemination of ***mycobacteria*** in host tissues are needed for a more complete understanding of the pathogenesis of diseases caused by

these bacteria and for the development of effective vaccines. Previous investigations have demonstrated that a 28-kDa heparin-binding ***mycobacterial*** surface protein, ***HBHA*** , can agglutinate erythrocytes and promote ***mycobacterial*** aggregation in vitro. In this study, further molecular and biochemical analysis of ***HBHA*** demonstrates that it has three functional domains: a transmembrane domain of 18 amino acids residing near the N terminus, a. . . acids consistent with an alpha-helical coiled-coil region, and a Lys-Pro-Ala-rich C-terminal domain that mediates binding to proteoglycans. Using His-tagged recombinant ***HBHA*** proteins and nickel chromatography we demonstrate that ***HBHA*** polypeptides which contain the coiled-coil region form multimers. This tendency to oligomerize may be responsible for the induction of ***mycobacterial*** aggregation since a truncated N-terminal ***HBHA*** fragment containing the coiled-coil domain promotes ***mycobacterial*** aggregation. Conversely, a truncated C-terminal ***HBHA*** fragment which contains Lys-Pro-Ala-rich repeats binds to the proteoglycan decorin. These results indicate that ***HBHA*** contains at least three distinct domains which facilitate intercalation into surface membranes, promote bacterium-bacterium interactions, and mediate the attachment to. . .

BC ***Mycobacteriaceae*** 08881

IT . . .
Biochemistry and Molecular Biophysics; Infection

IT Parts, Structures, & Systems of Organisms
erythrocytes: blood and lymphatics

IT Chemicals & Biochemicals
HBHA ; hemagglutinin: functional domains

IT Methods & Equipment
biochemical analysis: analytical method; nickel chromatography: analytical method

IT Miscellaneous Descriptors
mycobacterial aggregation

ORGN Super Taxa
Mycobacteriaceae : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
mycobacteria (***Mycobacteriaceae***): pathogen

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L6 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 6

AN 1999:445145 BIOSIS

DN PREV199900445145

TI Immunogenicity of DNA vaccines expressing tuberculosis proteins fused to tissue plasminogen activator signal sequences.

AU Li, Zhongming; Howard, Angela; Kelley, Cynthia; Delogu, Giovanni; Collins, Frank; Morris, Sheldon (1)

CS (1) Laboratory of Mycobacteria, OVRR/CBER/FDA, HFM-431, 29 Lincoln Dr., Building 29, Room 502, Bethesda, MD, 20892 USA

SO Infection and Immunity, (Sept., 1999) Vol. 67, No. 9, pp. 4780-4786. ISSN: 0019-9567.

DT Article

LA English

SL English

AB Novel tuberculosis DNA vaccines encoding native ESAT-6, MPT-64, KatG, or

HBHA ***mycobacterial*** proteins or the same proteins fused to tissue plasminogen activator (TPA) signal sequences were evaluated for their capacity to elicit humoral, cell-mediated, and protective immune responses in vaccinated mice. While all eight plasmids induced specific humoral responses, the constructs expressing the TPA fusions generally evoked higher antibody responses in vaccinated hosts. Although most of the DNA vaccines tested induced a substantial gamma interferon response in the spleen, the antigen-specific lung responses were 2- to 10-fold lower than the splenic responses at the time of challenge. DNA vaccines encoding the ESAT-6, MPT-64, and KatG antigens fused to TPA signal sequences evoked significant protective responses in mice aerogenically challenged with low doses of ***Mycobacterium*** tuberculosis Erdman 17 to 21 days after the final immunization. However, the protective response induced by live ***Mycobacterium*** bovis BCG vaccine was greater than the response induced by any of the DNA vaccines tested. These results suggest that the tuberculosis DNA vaccines were able to elicit substantial immune responses in suitably vaccinated mice, but further refinements to the constructs or the use of alternative immunization strategies will be needed to improve the efficacy of these vaccine candidates.

AB Novel tuberculosis DNA vaccines encoding native ESAT-6, MPT-64, KatG, or ***HBHA*** ***mycobacterial*** proteins or the same proteins fused to tissue plasminogen activator (TPA) signal sequences were evaluated for their capacity to elicit. . . and KatG antigens fused to TPA signal sequences evoked significant protective responses in mice aerogenically challenged with low doses of ***Mycobacterium*** tuberculosis Erdman 17 to 21 days after the final immunization. However, the protective response induced by live ***Mycobacterium*** bovis BCG vaccine was greater than the response induced by any of the DNA vaccines tested. These results suggest that. . .

BC ***Mycobacteriaceae*** 08881
Muridae 86375

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Infection; Pharmacology

IT Chemicals & Biochemicals
tissue plasminogen activator: signal sequence; BCG: ***Mycobacterium*** bovis, vaccine; DNA vaccine: immunogenicity, vaccine; Erdman: ***Mycobacterium*** tuberculosis, vaccine; ESAT-6: ***mycobacterial*** protein; ***HBHA*** : ***mycobacterial*** protein; KatG: ***mycobacterial*** protein; MPT-64: ***mycobacterial*** protein

ORGN Super Taxa
Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; ***Mycobacteriaceae*** : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
mouse (Muridae); ***Mycobacterium*** bovis (***Mycobacteriaceae***): pathogen; ***Mycobacterium*** tuberculosis (***Mycobacteriaceae***): pathogen

ORGN Organism Superterms
Animals; Bacteria; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

L6 ANSWER 8 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 7
AN 1998:497675 BIOSIS

DN PREV199800497675

TI Molecular characterization of the ***mycobacterial*** heparin-binding hemagglutinin, a ***mycobacterial*** adhesin.

AU Menozzi, Franco D.; Bischoff, Rainer; Fort, Emmanuelle; Brennan, Michael J.; Locht, Camile (1)

CS (1) Lab. Microbiol., Genet. Mol., Inst. Natl. Sante Recherche Med. U447, Inst. Pasteur Lille, 1 rue Calmette, F-59019 Lille Cedex France

SO Proceedings of the National Academy of Sciences of the United States of America, (Oct. 13, 1998) Vol. 95, No. 21, pp. 12625-12630. ISSN: 0027-8424.

DT Article

LA English

AB Although it generally is accepted that the interaction of ***Mycobacterium*** tuberculosis with alveolar macrophages is a key step in the pathogenesis of tuberculosis, interactions with other cell types, especially epithelia(cells, also may be important. In this study we describe the molecular characterization of a ***mycobacterial*** heparin-binding hemagglutinin (***HBHA***), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from M. tuberculosis and bacillus Calmette-Guerin, and the sequence was found to be identical between the two species. The calculated M, was smaller than the observed Mr when analyzed by SDS/PAGE. This difference can be attributed to the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of ***HBHA*** appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of ***HBHA*** to sulfated carbohydrates. Evidence suggests that glycosylation is also important for ***HBHA*** -mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of ***HBHA*** raises the possibility that this protein is not secreted via the general secretion pathway.

TI Molecular characterization of the ***mycobacterial*** heparin-binding hemagglutinin, a ***mycobacterial*** adhesin.

AB Although it generally is accepted that the interaction of ***Mycobacterium*** tuberculosis with alveolar macrophages is a key step in the pathogenesis of tuberculosis, interactions with other cell types, especially epithelia(cells, also may be important. In this study we describe the molecular characterization of a ***mycobacterial*** heparin-binding hemagglutinin (***HBHA***), a protein that functions as an adhesin for epithelial cells. The structural gene was cloned from M. tuberculosis and bacillus. . . the Lys/Pro-rich repeats that occur at the C-terminal end of the protein and to a putative carbohydrate moiety. Glycosylation of ***HBHA*** appears to protect the protein from proteolytic degradation, which results in the removal of the C-terminal Lys/Pro-rich region responsible for binding of ***HBHA*** to sulfated carbohydrates. Evidence suggests that glycosylation is also important for ***HBHA*** -mediated hemagglutination and for certain immunologic properties of the protein. Finally, the absence of a signal peptide in the coding region of ***HBHA*** raises the possibility that this protein is not secreted via the general secretion pathway.

BC ***Mycobacteriaceae*** 08881

IT Major Concepts

Infection; Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Diseases

tuberculosis: bacterial disease
IT Chemicals & Biochemicals
mycobacterial heparin-binding hemagglutinin: adhesin, secretion, glycosylation
ORGN Super Taxa
Mycobacteriaceae : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Mycobacterium -tuberculosis (***Mycobacteriaceae***): pathogen
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L6 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS
AN 1998:418247 BIOSIS
DN PREV199800418247
TI Multimeric properties of a ***mycobacterial*** adhesin.
AU Delogu, G.; Brennan, M. J.
CS CBER, FDA, Bethesda, MD USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1998) Vol. 98, pp. 510.
Meeting Info.: 98th General Meeting of the American Society for Microbiology Atlanta, Georgia, USA May 17-21, 1998 American Society for Microbiology
. ISSN: 1060-2011.
DT Conference
LA English
TI Multimeric properties of a ***mycobacterial*** adhesin.
BC ***Mycobacteriaceae*** 08881
IT Major Concepts
Biochemistry and Molecular Biophysics
IT Chemicals & Biochemicals
HBHA : adhesin, function, multimer
ORGN Super Taxa
Mycobacteriaceae : ***Mycobacteria*** , Actinomycetes and Related Organisms, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
mycobacteria (***Mycobacteriaceae***);
Mycobacterium -tuberculosis (***Mycobacteriaceae***)
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L6 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 1997:776266 CAPLUS
DN 128:58314
TI Cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection
IN Menozzi, Franco; Locht, Camille
PA Institut Pasteur De Lille, Fr.; Institut National De La Sante Et De La Recherche Medicale; Menozzi, Franco; Locht, Camille
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent
LA French

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9744463	A2	19971127	WO 1997-FR886	19970520
WO 9744463	A3	19980129		
W: AU, CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2748748	A1	19971121	FR 1996-6168	19960517
FR 2748748	B1	19981106		
AU 9730362	A1	19971209	AU 1997-30362	19970520
EP 914437	A2	19990512	EP 1997-925109	19970520
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI FR 1996-6168		19960517		
WO 1997-FR886		19970520		

AB The invention features proteins enabling ***mycobacteria*** adhesion to host cells, in particular to epithelial cells. More particularly, the invention features a ***mycobacterial*** antigen of the heparin-binding hemagglutinin type (***HBHA***) obtained from ***Mycobacterium*** bovis BCG or M. tuberculosis. The invention particularly features the expression product of an Escherichia coli strain transformed with an ***HBHA*** gene. These protein sequences can be used in immunogenic compns., for prepg. vaccines against ***mycobacterial*** infections, and for diagnosis of ***mycobacterial*** infection. Addnl., use of sulfated carbohydrates (such as heparin, chondroitin sulfate, dextran sulfate) to prevent adhesion of ***mycobacteria*** to epithelial cells is claimed. Sera of tuberculosis patients contain antibodies to ***HBHA*** while sera of healthy people do not. The post-translational modification of native ***HBHA*** (presumably glycosylation) was shown to provide a major antigenic determinant of ***HBHA*** .

TI Cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection

AB The invention features proteins enabling ***mycobacteria*** adhesion to host cells, in particular to epithelial cells. More particularly, the invention features a ***mycobacterial*** antigen of the heparin-binding hemagglutinin type (***HBHA***) obtained from ***Mycobacterium*** bovis BCG or M. tuberculosis. The invention particularly features the expression product of an Escherichia coli strain transformed with an ***HBHA*** gene. These protein sequences can be used in immunogenic compns., for prepg. vaccines against ***mycobacterial*** infections, and for diagnosis of ***mycobacterial*** infection. Addnl., use of sulfated carbohydrates (such as heparin, chondroitin sulfate, dextran sulfate) to prevent adhesion of ***mycobacteria*** to epithelial cells is claimed. Sera of tuberculosis patients contain antibodies to ***HBHA*** while sera of healthy people do not. The post-translational modification of native ***HBHA*** (presumably glycosylation) was shown to provide a major antigenic determinant of ***HBHA*** .

ST sequence ***Mycobacterium*** heparin binding hemagglutinin gene; vaccine diagnosis ***Mycobacterium*** infection

IT Antibodies

RL: ANT (Analyte); ANST (Analytical study)

(anti-hemagglutinin; cloning and expression of ***mycobacterial***

heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT Adhesion (biological)
Diagnosis
Epithelium
Mycobacterium
Mycobacterium BCG
Mycobacterium tuberculosis
Vaccines
(cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT Escherichia coli
(hemagglutinin prodn. with recombinant; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT Antitumor agents
(hemagglutinin-producing cells as; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT Hemagglutinins
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(heparin-binding; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT DNA sequences
(of heparin-binding hemagglutinin gene of ***Mycobacterium*** bovis BCG)

IT Protein sequences
(of heparin-binding hemagglutinin of ***Mycobacterium*** bovis BCG)

IT Carbohydrates, biological studies
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(sulfates, inhibition of ***mycobacterial*** adhesion with; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT 200295-64-3
RL: PRP (Properties)
(C-terminus of hemagglutinin; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT 200361-22-4
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT 9005-49-6, Heparin, biological studies 9007-28-7, Chondroitin sulfate 9042-14-2, Dextran sulfate 9072-19-9, Fucoidin
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(inhibition of ***mycobacterial*** adhesion with; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin

gene and vaccination against and diagnosis of ***mycobacterial*** infection)

IT 200361-21-3
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; cloning and expression of ***mycobacterial*** heparin-binding hemagglutinin gene and vaccination against and diagnosis of ***mycobacterial*** infection)

L6 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 8
AN 1996:510320 BIOSIS
DN PREV199699232676
TI Identification of a heparin-binding hemagglutinin present in ***mycobacteria*** .
AU Menozzi, Franco D.; Rouse, Julie H.; Alavi, Mohammad; Laude-Sharp, Marilyn; Muller, Jacqueline; Bischoff, Rainer; Brennan, Michael J. (1); Locht, Camille
CS (1) CBER/FDA, 1401 Rockville Pike (HFM-431), Rockville, MD 20852-1448 USA
SO Journal of Experimental Medicine, (1996) Vol. 184, No. 3, pp. 993-1001. ISSN: 0022-1007.
DT Article
LA English
AB Adherence to mammalian host tissues is an important virulence trait in microbial pathogenesis, yet little is known about the adherence mechanisms of ***mycobacteria*** . Here, we show that binding of ***mycobacteria*** to epithelial cells but not to macrophages can be specifically inhibited by sulfated carbohydrates. Using heparin-Sepharose chromatography, a 28-kD heparin-binding protein was purified from culture supernatants and cell extracts of ***Mycobacterium*** bovis and ***Mycobacterium*** tuberculosis. This protein, designated heparin-binding hemagglutinin (***HBHA***), promotes the agglutination of rabbit erythrocytes, which is specifically inhibited by sulfated carbohydrates. ***HBHA*** also induces ***mycobacterial*** aggregation, suggesting that it can mediate bacteria-bacteria interactions as well. Hemagglutination, ***mycobacterial*** aggregation, as well as attachment to epithelial cells are specifically inhibited in the presence of anti- ***HBHA*** antibodies. Immunoelectron microscopy using anti- ***HBHA*** monoclonal antibodies revealed that the protein is surface exposed, consistent with a role in adherence. Immunoblot analyses using antigen-specific antibodies indicated that ***HBHA*** is different from the fibronectin-binding proteins of the antigen 85 complex and p55, and comparison of the NH-2-terminal amino acid sequence of purified ***HBHA*** with the protein sequence data bases did not reveal any significant similarity with other known proteins. Sera from tuberculosis patients but not from healthy individuals were found to recognize ***HBHA*** , indicating its immunogenicity in humans during ***mycobacterial*** infections. Identification of putative ***mycobacterial*** adhesins, such as the one described in this report, may provide the basis for the development of new therapeutic and prophylactic strategies against ***mycobacterial*** diseases.
TI Identification of a heparin-binding hemagglutinin present in ***mycobacteria*** .
AB. . . mammalian host tissues is an important virulence trait in microbial pathogenesis, yet little is known about the adherence mechanisms of ***mycobacteria*** . Here, we show that binding of ***mycobacteria***

to epithelial cells but not to macrophages can be specifically inhibited by sulfated carbohydrates. Using heparin-Sepharose chromatography, a 28-kD heparin-binding protein was purified from culture supernatants and cell extracts of ***Mycobacterium*** bovis and ***Mycobacterium*** tuberculosis. This protein, designated heparin-binding hemagglutinin (***HBHA***), promotes the agglutination of rabbit erythrocytes, which is specifically inhibited by sulfated carbohydrates. ***HBHA*** also induces ***mycobacterial*** aggregation, suggesting that it can mediate bacteria-bacteria interactions as well. Hemagglutination, ***mycobacterial*** aggregation, as well as attachment to epithelial cells are specifically inhibited in the presence of anti- ***HBHA*** antibodies. Immunoelectron microscopy using anti- ***HBHA*** monoclonal antibodies revealed that the protein is surface exposed, consistent with a role in adherence. Immunoblot analyses using antigen-specific antibodies indicated that ***HBHA*** is different from the fibronectin-binding proteins of the antigen 85 complex and p55, and comparison of the NH-2-terminal amino acid sequence of purified ***HBHA*** with the protein sequence data bases did not reveal any significant similarity with other known proteins. Sera from tuberculosis patients but not from healthy individuals were found to recognize ***HBHA*** , indicating its immunogenicity in humans during ***mycobacterial*** infections. Identification of putative ***mycobacterial*** adhesins, such as the one described in this report, may provide the basis for the development of new therapeutic and prophylactic strategies against ***mycobacterial*** diseases.

BC ***Mycobacteriaceae*** 08881
Leporidae 86040
Hominidae *86215

IT Miscellaneous Descriptors
BACTERIAL DISEASE; EPITHELIAL CELL; ERYTHROCYTES; HEPARIN-BINDING HEMAGGLUTININ; HEPARIN-SEPHAROSE CHROMATOGRAPHY; INFECTION; ***MYCOBACTERIAL*** INFECTIONS; ***MYCOBACTERIUM*** -TUBERCULOSIS; PATIENT; PURIFICATION METHOD

ORGN Super Taxa
Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia;
Leporidae: Lagomorpha, Mammalia, Vertebrata, Chordata, Animalia;
Mycobacteriaceae : Eubacteria, Bacteria

ORGN Organism Name
human (Hominidae); rabbit (Leporidae); ***Mycobacterium*** bovis (***Mycobacteriaceae***)

ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; lagomorphs; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; primates; vertebrates